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Thripinema nicklewoodi (Tylenchida: Allantonematidae), a potential biological control agent of Frankliniella occidentalis (Thysanoptera: Thripidae).

Un Taek Lim
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THRIPINEMA NICKLEWOODI (TYLENCHIDA: ALLANTONEMATIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF *FRANKLINIELLA OCCIDENTALIS* (THYSANOPTERA: THRIPIDAE)

A Dissertation Presented

by

UN TAEK LIM

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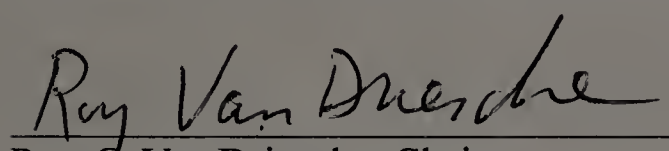

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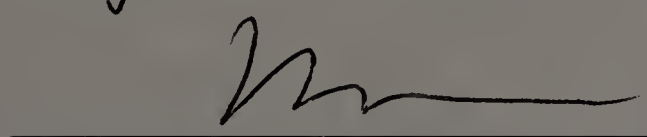

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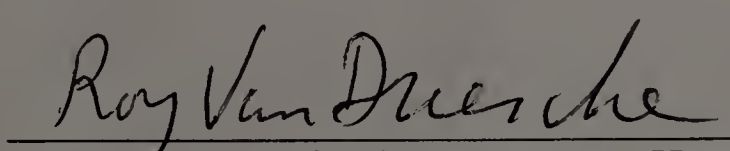

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DEDICATION

To my loving family.

ACKNOWLEDGMENTS

I want to give my special thanks to Dr. Roy Van Driesche, my advisor, who guided and supported me throughout my Ph. D. study. I respect his thoughtful consideration for his students as well as his passion for the field of biological control. The results presented here would not be possible without his sincere guidance and knowledge I learned from him, and I will never forget what he has done for me.

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ABSTRACT

THRIPINEMA NICKLEWOODI (TYLENCHIDA: ALLANTONEMATIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF *FRANKLINIELLA OCCIDENTALIS* (THYSANOPTERA: THRIPIDAE)

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Potential of *Thripinema nicklewoodi* Siddiqi (Tylenchida: Allantonematidae) as a biological control agent of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) was studied.

I developed methods to propagate and study *T. nicklewoodi*. I observed an excretion rate of 21.4 nematodes per day by parasitized female thrips. The sex ratio of the excreted nematodes was 6: 1 (female: male). After exposing 50 healthy first instars to four parasitized female thrips in a rolled bean leaf, I obtained a 75.3% mean parasitization rate in the adult stage of the thrips. In contrast to previous reports, male thrips were found to be parasitized as readily as females. Parasitism reduced the longevity of both adult female and male thrips by 26% and 61%, respectively. *T.*

nicklewoodi, when presented with various thrips life stages, achieved the highest attack rate in first and second instars and prepupa. Free-living nematodes were found to escape from hosts through the anus and penetrate new host thrips through the intersegmental membranes of the thorax and abdomen.

While nematode parasitization affected tospovirus propagation, it did not reduce virus transmission even though parasitism reduced feeding activity of adult female thrips by 81% on leaves, 38% on pollen, and 22% on honey. However, despite lowered total feeding, probing by parasitized thrips (in honey) was not reduced, and this may explain why lowered feeding does not result in lowered virus transmission.

In a study of the population dynamics on caged impatiens in a greenhouse, nematode transmission persisted for seven host generations and populations of healthy (i.e., not parasitized) female thrips declined by 39-79% in the nematode treatment compared to the control. However, no significant reductions were found in numbers of larval thrips between nematode treatments and the control. A higher proportion of male thrips occurred in populations with nematodes in which adult female thrips declined significantly compared to the control population.

T. nicklewoodi released seven times on caged impatiens in a greenhouse did not provide preventative control of thrips, though the population growth of second instar, adult female, and male thrips was suppressed by 44, 68, and 49%, respectively.

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CHAPTER I

BIOLOGICAL ATTRIBUTES OF NEMATODE

Introduction

Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae), is a serious pest of many field and greenhouse crops worldwide (Robb, 1989). This pest species reduces the productivity of crops in two ways: direct feeding and serving as a vector for plant viruses such as impatiens necrotic spot virus (INSV) and tomato spotted wilt virus (TSWV). *Frankliniella occidentalis* is difficult to control because of its small size, rapid reproductive rate, and preference to feed within flowers and buds. Furthermore, control with many insecticides is often poor, in part, due to pesticide resistance (Robb, 1989; Immaraju *et al.*, 1992). Although several predators and parasites have been considered for biological control of thrips and some are available commercially, they have only a limited ability to reduce thrips populations. The arthropod natural enemies used against thrips are apparently hindered from entering tight flower buds, meristematic tissues, or narrow flower structures due to their relatively large body size, hence limiting their ability to reduce thrips populations in their preferred microhabitat (Loomans *et al.*, 1995).

To overcome these problems, entomopathogenic nematodes, such as species in the genera *Steinernema* and *Heterorhabditis*, have been tried as biological control

agents, and some species have shown high attack rates in thrips (Helyer *et al.*, 1995; Ebssa *et al.*, 2001). But efficiency of these nematodes is also limited because they can only parasitize thrips life stage dwelling in the soil. However, the entomoparasitic nematode *Thripinema nicklewoodi* Siddiqi (Tylenchida: Allantonematidae) may be a more effective biological control agent because it has a different mode of action. Parasitization by this nematode does not kill the host thrips immediately. Instead, the larvae live to become adults that do not produce eggs but do vector the nematodes in their feces into flowers and buds where thrips congregate. The ovaries of thrips parasitized by *T. nicklewoodi* were reported to be greatly reduced (Nickle and Wood, 1964).

T. nicklewoodi was first described in 1964 as *Howardula aptini* Sharga (Siddiqi, 1985). Eight species of thrips are known hosts of nematodes in the genus *Thripinema*: *Aptinothrips rufus* Gmelin (Sharga, 1932), *F. vaccinii* Morgan and *Taeniothrips vaceinoptilus* Hood (Nickle and Wood, 1964), *F. occidentalis* (Wilson and Cooley, 1972), *Megaluriothrips sp.* (Reddy *et al.*, 1982), *Microcephalothrips abdominalis* Crawford and *F. schultzei* Trybom (Varatharajan, 1985), *Thrips obscuratus* Crawford (Teulon *et al.*, 1997), *F. fusca* (Tipping *et al.*, 1998), and *F. australis* (Funderburk *et al.*, 2002b). Greene and Parrella (1993) reported that 19-33% of *F. occidentalis* collected in California were parasitized by *T. nicklewoodi*. Heinz *et al.* (1996) reported that *T. nicklewoodi* exhibited a positive association with *F. occidentalis* densities after surveying field- and greenhouse-grown carnations, chrysanthemums, and roses throughout California to identify potentially effective

natural enemies of the thrips. However, the biology of this species of nematode and its interactions with *F. occidentalis*, especially the host searching behavior, are poorly known.

In this chapter, I describe a rearing method for *T. nicklewoodi* and a method for directly observing the interaction of the entomoparasitic nematode and its host. I used these methods to investigate the nematode excretion rate of hosts, abundance of nematodes in adult thrips, impact of inoculum load and host life stage on parasitization rates by *T. nicklewoodi* under laboratory conditions, effect of parasitization on the longevity of adult thrips, and host preference of nematode among thrips life stages. I also observed the host searching behavior. These factors provide a basis for better understanding the impact of *T. nicklewoodi* on *F. occidentalis* at the population level. This information is needed to support the assessment of the value of *T. nicklewoodi* for control of *F. occidentalis* in commercial greenhouses floral and bedding plant crops.

Materials and Methods

Source of Thrips and Nematode

Frankliniella occidentalis and *T. nicklewoodi* collected from alfalfa at the University of California at Davis Campus, California in 1998 by Kevin Heinz of Texas A&M University were sent to my laboratory where hosts and nematodes were cultured

at the Department of Entomology, University of Massachusetts, Amherst, Massachusetts. Thrips and nematodes used in all experiments were taken from these colonies.

Thrips Rearing

Frankliniella occidentalis were reared in plastic containers (19.7 × 19.4 × 7.14 cm, #3871, Rubbermaid, Wooster, OH) with 8 excised, rooted, pre-trifoliate bean leaves grown from food grade red kidney beans (*Phaseolus vulgaris* L.) per box, an approach modified from the method of Doane *et al.* (1995). A mixture of pure honey and castor bean (*Ricinus communis* L.) pollen was streaked along the main vein of each leaf. For ventilation, a 9 × 9 cm hole was cut in the lid of the container and covered with fine-mesh screen (95 μm diameter opening size, 3-95/38 NITEX, Sefar America INC, Kansas City, MO) attached to the lid with hot-melt glue. SuperMaxi feminine napkins (Personal Products Company, Skillman, NJ) soaked with tap water were used as a rooting medium for the leaves. From 25 to 30 newly emerged adult thrips were transferred to each new container. In rearing and all subsequent experiment, the thrips and nematodes were incubated at 24 °C, 50-60% RH, and 14L: 10D photoperiod in a growth chamber. Each container produced a new generation of thrips in approximately 3 weeks without changing the leaves. Life stages of thrips occurred mostly on the leaves, except pupae, which were found both on leaves and substrate. With this method, a cohort of first instars can be produced for use in parasitization trials in 6 days.

Nematode Rearing ("rolled leaf method")

Parasitized *F. occidentalis*, which yield the parasitic stage of the nematode, were used as the nematode source in all experiments and for propagation of the nematode colony. Thrips were recognized as parasitized by observing free living nematodes in feces excreted by the host when held between two sterile glass microscope slides (Fig. 1). Slides were held apart using a paper frame as a spacer. This spacer was made by folding a sheet of Kimwipes (Kimberly-Clark Corporation, Roswell, GA) four times, and cutting a hole in it with a paper-punch. The spacer was soaked with distilled water and placed on a slide glass. A leaf disk of red kidney bean was placed within the area framed by this paper spacer and the other glass slide was placed on top, after placing a thrips inside the framed area. The glass slides were then taped together at both ends and the confined thrips were incubated under the same conditions described in the rearing of thrips. These glass slide arenas retained enough humidity to prevent evaporation of feces droplets on the slide surfaces. Observations were made under a dissecting microscope.

To propagate a nematode colony, three or four parasitized adult female thrips (after their parasitization status was confirmed) were placed on a rolled red kidney bean leaf in the same plastic containers used in rearing thrips, together with 50 healthy first instar thrips. Leaves were trimmed to a standard dimension (5 × 8 cm) and streaked with honey and pollen along the midrib (about 3 cm) on the underside. The leaf blade was then rolled up, and tied with a cotton thread. The leaf petiole was then

inserted in a SuperMaxi feminine napkin soaked with water and used as a rooting medium for the leaf. The inside of the rolled leaf provided adequate humidity for survival while nematodes located new hosts. After 10 days of incubation, all adult thrips that developed from the larvae were collected and placed individually in glass slide arenas to determine whether or not they were parasitized. Parasitized thrips were set aside individually for use in subsequent experiments.

Exp. 1. Nematode Excretion Rate

To assess the rate of *T. nicklewoodi* excretion by parasitized thrips, I counted the daily number of nematodes produced by 46 parasitized adult female thrips obtained from the laboratory colony. Parasitized thrips were obtained by examining the colony rearing boxes daily to detect newly emerged adult thrips. These thrips were collected and confined individually in glass slide arenas to detect nematodes in thrips feces. Confined thrips was checked daily under a dissecting microscope, and those parasitized with nematodes were retained and observed until death. Nematodes excreted by these 46 female adults were counted daily and thrips were moved after each observation to a new glass slide arena. Thrips survived a maximum of 4 days under the experimental conditions. Numbers of nematodes excreted per thrips per day were compared among days from 1 to 4 days after first excretion with analysis of variance (ANOVA) using GLM procedure (SAS Institute, 1995). The sex ratio of 408 nematodes in each of 110 feces droplets was determined and the mean sex ratio calculated.

Exp. 2. Abundance of Nematodes in Parasitized Thrips

To determine the abundance of nematodes in parasitized thrips, 113 adult female and 131 male parasitized thrips were obtained from the laboratory nematode colony and dissected. In each parasitized thrips, I counted the total number of nematodes, including adult females and their progeny (i.e., eggs, juveniles, and parasitic adults of the second generation). I recognized adult females of the first generation based on their ovoid shape and shorter bodies compared to adults of the second generation (Reddy *et al.*, 1982). The numbers of second generation nematodes found in adult thrips were compared by two-way ANOVA using GLM procedure to identify significant effects of number of first generation nematodes and of host sex (SAS Institute, 1995). The mean numbers of second generation nematodes in each sex of thrips were further analyzed using Tukey's studentized range honestly significant difference (HSD) test.

Exp. 3. Dose Response Relationship

Using the rolled leaf method employed to rear *T. nicklewoodi*, I assessed the dose relationship between the number of parasitized adult female thrips confined with healthy first instar thrips and the resultant parasitization rate in these larvae after they had reached the adult stage. Either 0 (control), 1, 2, 3, or 4 parasitized adult female thrips were placed on a rolled red kidney bean leaf in the same type of plastic

container as used to rear thrips together with 50 healthy first instar thrips of undetermined sex ratio. Each treatment was replicated 10 times. Thrips were incubated for 10 days to allow sufficient time for all larval thrips to reach the adult stage. The proportion of surviving adult thrips parasitized by nematodes was determined by dissection and the sex of each thrips was noted. The parasitization rates of each sex in each replicate were arcsine transformed and analyzed with two-way ANOVA to identify significant dose and sex effects. Numbers of both female and male thrips in each container were also counted, and analyzed with one-way ANOVA to identify significant dose effect on the emergence of both female and male thrips (SAS Institute, 1995).

Exp. 4. Effect of Nematode Parasitization on the Longevity of Adult Thrips

One-day-old adult thrips (54 males and 122 females) from the laboratory nematode colony were isolated individually in plastic diet cups (29.6 ml capacity, Comet Products, Chelmsford, MA) and their daily survival monitored. These thrips had been exposed as larvae to nematodes, and an unknown proportion were expected to be parasitized. Thrips in cups were provided with a bean leaf disk (1.5×1.5 cm) streaked with a honey and pollen mixture as food. The leaf disk was placed on a folded Kimwipe soaked with distilled water. The food and cups were changed every 3 days. On the day each thrips died, it was dissected to determine its sex and if it was parasitized. The longevity of thrips was analyzed with two-way ANOVA using GLM

procedure to identify significant effect of sex and parasitization (SAS Institute, 1995). In addition, the survivorship curves for parasitized and healthy thrips of each sex were plotted.

Exp. 5. Host Preference of Nematode among Thrips Life Stages and Host Searching Behavior

Host life stage preference. I determined which life stages of the host were acceptable for parasitization by *T. nicklewoodi*, and which stages, if any, were preferred. This experiment was performed in an arena intended to imitate the space inside a flower, where *T. nicklewoodi* is believed to forage for thrips in nature. The arena provided enough humidity to support movement of the nematode and the limited space promoted aggregation of the thrips. The arena was constructed of two micropipette tips (200 µl capacity) as shown in Fig. 2. A parasitized adult female thrips was confined in this arena with six other thrips, one of each life stage except the egg: first instar, second instar, prepupa, pupa, adult male, and adult female. A leaf disk streaked with a honey-pollen mixture was provided as a food source. Thrips were held in the arena for 1-day in a growth chamber. I then opened the arena by removing one micropipette tip and collected all thrips. I then dissected all thrips collected from the micropipette tips, and nematodes found inside thrips were regarded as those that succeeded in parasitizing the host. I counted and sexed any nematodes found in the host. I replicated this experiment 30 times and estimated both the parasitization

frequency in each life stage and the total number of nematodes entering each thrips lifestage. The differences in parasitization among life stages were analyzed using Chi-square contingency tables (SAS Institute, 1995).

Nematode behavior toward host. I observed the host searching behavior of *T. nicklewoodi* under a dissecting microscope (25×). I used the same glass slide arena as used to observe nematodes for Exp. 1 (Fig. 1). After locating a free-living nematode on the inside surface of the glass slides or on the leaf disk, I observed the movement of the nematode.

Results

Exp. 1. Nematode Excretion Rate

I observed actively moving nematodes in thrips feces when the thrips were confined between glass microscope slides. The numbers of *T. nicklewoodi* excreted per adult female thrips per day were 13.6 (± 12.6 SD) in day 1, 22.6 (± 10.1 SD) in day 2, 23.5 (± 9.8 SD) in day 3, and 25.8 (± 12.8 SD) in day 4. The mean number was 21.4 (± 5.2 SD) during the 4 days, with differences among days in excretion rate being significant ($F_{3, 93} = 5.17$, $P = 0.002$). However, the numbers of nematode excreted on day 1 may represent less than a 24-h period. If data for day 1 are excluded, the mean daily excretion rate was 24.0 (± 1.6 SD) and no difference among days remains ($F_{2, 48}$

= 0.18, $P = 0.840$). The average sex ratio of groups of nematodes found in 110 feces droplets was 6.0: 1.0 (female: male), with one droplet containing an average of 3.2 (± 2.3 SD) females and 0.5 (± 0.7 SD) males.

Exp. 2. Abundance of Nematodes in Parasitized Thrips

I found a maximum of 11 ovoid-shape first generation nematodes in a female thrips and a maximum of 6 in a male. The number of second generation nematodes per host differed significantly between female and male thrips, and there was significant effect of number of first generation nematodes (two-way ANOVA; sex $F_{1, 234} = 127.31$, $P < 0.001$; number of first generation nematodes $F_{4, 234} = 7.23$, $P < 0.001$; interaction $F_{4, 234} = 6.64$, $P < 0.001$) (Fig. 3). The average number of second generation nematodes per host in female thrips was 192.6 (± 8.2 SE, n = 113) and in male thrips was 93.7 (± 2.2 SE, n = 131). The number of second generation nematodes in female hosts was lowest (144.5 ± 10.5 SE) in thrips with one first generation female nematode and highest (255.7 ± 45.9 SE) in thrips with four first generation female nematodes and varied significantly with the number of first generation nematodes ($F_{4, 108} = 7.06$, $P < 0.001$) (Fig. 3). In male thrips, however, the number of second generation nematodes did not vary with the number of first generation nematodes parasitizing a host ($F_{4, 126} = 0.98$, $P = 0.420$) (Fig. 3).

Exp. 3. Dose Response Relationship

The parasitization rate (measured after exposed larvae reached the adult stage) of both sexes combined increased steadily from 26.9% (± 15.6 SD), when 1 parasitized adult thrips was used as inoculum, to 75.3% (± 9.9 SD), when 4 were employed (Fig. 4). Male and female thrips were parasitized with equal frequency (two-way ANOVA; sex $F_{1, 90} = 0.97$, $P = 0.327$; inoculum level $F_{4, 90} = 53.72$, $P < 0.001$; interaction $F_{4, 90} = 0.16$, $P = 0.957$) (Fig. 4). Neither the numbers of surviving female thrips (from the first instar larvae to adults) nor of male thrips were different among the control and treated groups, regardless of the inoculum level, i.e., number of parasitized thrips used (female thrips $F_{4, 45} = 0.38$, $P = 0.820$; male thrips $F_{4, 45} = 1.22$, $P = 0.318$) (Fig. 5).

Exp. 4. Effect of Nematode Parasitization on the Longevity of Adult Thrips

There was no difference in longevity between healthy female and male thrips, but parasitization significantly reduced the longevity of both adult male and female thrips. The degree of reduction in longevity was larger in parasitized males (61%) than in parasitized females (26%) (two-way ANOVA; sex $F_{1, 172} = 1.29$, $P = 0.258$; parasitization $F_{1, 172} = 35.69$, $P < 0.001$; interaction $F_{1, 172} = 7.56$, $P = 0.007$) (Table 1). Mortality of parasitized male thrips occurred at an earlier age compared to that of females (Fig. 6).

Exp. 5. Host Preference of Nematode among Thrips Life Stages and Host Searching Behavior

Host life stage preference. *T. nicklewoodi* parasitized all life stages of *F. occidentalis* tested. Thrips of different life stages were attacked at significantly different rates ($\chi^2 = 34.75$, d.f. = 5, $P < 0.001$). The highest frequency of parasitization was found in second instar larvae (Table 2), and in general younger life stages were parasitized at higher rates than older life stages. Although the sex ratio of excreted nematodes was found to be 6: 1 (female: male) in Exp. 1, of 131 nematodes recovered after they entered a host, only 2 nematodes were male. The maximum number of nematodes entering a single host during a 1-day exposure period was 5, which was found once each in a first instar, a second instar, and a prepupa.

Nematode behavior toward host. The free-living nematodes escaped the hosts through the anus (Figs. 7 and 8) actively migrated to a trichome on leaf disks in the observation arenas and moved up this structure. Then, the nematode actively waved the anterior part of its body while attaching itself to the trichome with the posterior part of its body. After a nematode contacted the leg of a thrips, the nematode immediately moved up along the leg toward the abdomen of the host and penetrated an intersegmental membrane (Fig. 9). I also photographed the nematode penetrating through coxal cavity (Fig. 10).

Discussion

I found first that male thrips can be parasitized by *T. nicklewoodi* in contrast to earlier studies in which only females were observed to be parasitized (Wilson and Cooley, 1972; Varatharajan, 1985; Greene and Parrella, 1993; Teulon *et al.* 1997), and this observation is also supported by work of Mason and Heinz (2002). I found no significant difference in the parasitization rate of thrips for adult males and females (Fig. 4). Previous studies may have failed to observe parasitization of male thrips because the sex ratio of thrips and related species is strongly female biased, although the degree of this bias varies seasonally. Also parasitization by *T. nicklewoodi* reduces the longevity of the adult male thrips to a greater degree than for females (Table 1 and see also below for details).

Secondly, I showed that the daily nematode-excretion rate is relatively constant. This constant excretion rate may be related to the host diuretic behavior. I do not know whether parasitization increases the fecal excretion rate of the host or not. The constant daily nematode excretion rate is consistent with the observation of a steady increase in parasitization rate in the dose response experiment (Fig. 4) as the number of parasitized adult thrips used as inoculum was increased.

Thirdly, I found that the majority (99%) of nematodes entering hosts were female (Exp. 5), with the sex ratio of newly excreted nematodes being strongly biased female (86%) (Exp. 1). This suggests that nematodes are likely to have mated before entering new host. I also found more nematodes in adult female thrips than male thrips, and this may be related to the relatively large body size of adult female thrips.

Fourthly, I showed that parasitization by *T. nicklewoodi* has no harmful effect on survival of parasitized thrips from early in the first instar to the emergence of the adult thrips and does not affect the sex ratio of the resulting adults (Fig. 5). In the adult stage, parasitization reduces the survival of both sexes of *F. occidentalis*, but longevity of the female is still approximately 74% of healthy thrips. This is important as survival of parasitized females for a prolonged period is key to the reproduction and efficient transmission of nematodes to larval thrips at feeding sites. Given an excretion rate of 21.4 nematodes per day by female thrips, parasitized females could excrete up to 250 nematodes in their lifetime. The effect of nematode parasitization on longevity of the adult male thrips, however, is more detrimental. Male thrips feed less often than females (van de Wetering *et al.*, 1998), and consequently nematodes in male thrips may deplete body fluids of male hosts more quickly, reducing their longevity. This may explain the failure of past studies to detect parasitization of field-collected male thrips.

Finally, I found that thrips life stages vary in their susceptibility to be parasitized. The low parasitization rates that I detected in the older life stages, i.e., pupa, adult female and adult male, may be related to the hardening of the cuticle as thrips age and the need for younger hosts for the successful development and spread of the nematode.

Based on this study, I summarize the process of thrips parasitization by the *T. nicklewoodii* as follows. Nematodes of the second generation escape from the adult thrips host through anus. Nematode have a 6: 1 (female to male) sex ratio and female nematode may have mated before entering new host. Escaped nematodes actively

move toward trichome-like structures of the plant leaf or flower petal and climb those structures if they are met. Then, nematodes actively rotate the anterior part of the body while fixing the posterior part to the trichome-like structures to make random contact with thrips on legs or other structures. If nematode succeeds in contacting the body of a host, they immediately move up the body until they reach the host's abdomen or coxal cavity. Successful parasitization is highest in younger stages of the thrips, and nematodes do not have a preference for the sex of the host. After entering the new host, the female nematode becomes a swollen sac-like organism. The number of the eggs laid by the female nematode is affected by the host sex and the presence of other nematodes present in the same host. The nematodes emerging from the eggs develop into adults, which are ready to escape the host for the next life cycle.

CHAPTER II

EFFECT OF NEMATODE PARASITIZATION ON TOSPOVIRUS TRANSMISSION BY THRIPS

Introduction

Frankliniella occidentalis Pergande (Thysanoptera: Thripidae), a local pest in the western USA until about 1980, has become a major pest worldwide on many crops and wild plants (Yudin et al., 1986; Robb, 1989; Shipp and Zariffa, 1991; Daughtrey et al., 1997; Jacobson, 1997; Kiers et al., 2000). In addition to the direct feeding damage on plants, the tospoviruses transmitted by *F. occidentalis*, *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), *Groundnut ringspot virus* (GBNV), *Tomato chlorotic spot virus* (TCSV) and *Chrysanthemum stem necrosis virus* (CSNV), have caused significant crop losses (Nagata and Peters, 2001; Ullman et al., 2002). Among the tospoviruses, INSV in particular has become a major pathogen in greenhouse flower crops in North America and Europe (Daughtrey et al., 1997) after being found first in *Impatiens* sp. in the U.S.A. by Law and Moyer (1990).

Thripinema nicklewoodi Siddiqi (Tylenchida: Allantonematidae) has been proposed as a potential biological control agent of *F. occidentalis* (Greene and Parrella, 1993; Heinz et al., 1996; Arthurs and Heinz, 2002; Mason and Heinz, 2002). Different from commercially available species of *Steinernema* and *Heterorhabditis* nematodes, which can be applied against soil-dwelling life stages of thrips, i.e., late second instar

larvae, prepupae, and pupae (Ebssa et al., 2001), *T. nicklewoodi* can be used to attack all post-egg life stages of *F. occidentalis*, even the adult (Table 2; Mason and Heinz, 2002). Parasitization causes sterilization of *F. occidentalis*. Reduction of ovaries in parasitized thrips has been observed in *F. occidentalis* parasitized by *T. nicklewoodi*, and no or few thrips eggs were present in parasitized *F. occidentalis* (Nickle and Wood, 1964; Greene and Parrella, 1993). Sterilization has also been found in other thrips parasitized by various *Thripinema* species: *Aptinothrips rufus* parasitized by *T. aptini* (formerly *Tylenchus aptini*) (Sharga, 1932), *F. fusca* by *T. fuscum* (Tipping et al., 1998), *F. australis* by *T. khrustalevi* (Funderburk et al., 2002), and *Megaluriothrips* sp. by *T. reniraoi* (formerly *Howardula aptini*) (Reddy et al., 1982). Parasitization also reduces the longevity of both adult female and male *F. occidentalis* by 26% and 61%, respectively (Table 1). However, parasitized thrips show no obvious physical or behavioral signs of *Thripinema* parasitization (Loomans et al., 1997) although Sharga (1932) observed sluggish behavior in *A. rufus* parasitized by *T. aptini*, and altered host behaviors have been documented for other arthropod hosts parasitized by nematode parasites (Townson, 1970; Moore and Lasswell, 1986; Rowland and Lindsay, 1986; Benton and Pritchard, 1990; Vance, 1996).

Since young vermiform larvae of *Thripinema* nematodes feed on the thrips' internal fluid (Loomans et al., 1997), the risk that nematode parasitization may accelerate the spread of INSV by increasing the feeding activity of parasitized thrips in response to the nutritional deficit imposed by the parasite (Chapman, 1998; Benton and Pritchard, 1990; Noma and Stricker, 2000) is, at least potentially, a major concern in use of this nematode. Furthermore, there are few studies on the effects of nematode

parasitization on the tospovirus transmission by viruliferous thrips. Here, I report results of studies on the effects of nematode parasitization on the acquisition and transmission of INSV by *F. occidentalis* and on the feeding behavior of the thrips.

Materials and Methods

INSV Culture

Impatiens plants (*Impatiens walleriana* Hook. f., var. 'Super Elfin Red') showing INSV symptoms were purchased from a local grower located in Amherst, Massachusetts and tested for INSV by Enzyme-linked Immunosorbent Assay (ELISA) kit (PSP 20500/0480, Agdia, Elkhart, Indiana). The procedures were followed the instruction manual provided by Agdia. Leaves of the impatiens plant that responded positively in the ELISA were cut and kept alive by inserting the petiole into water-soaked cotton in a small diet cup. Leaves were then fed to first instar larvae thrips for virus acquisition. The thrips were then held at 24 °C in a growth chamber for development. After adult emergence, the thrips were placed on two leaves of virus-free impatiens plants (*I. walleriana*, 'Baby Orange') grown from seeds (The Chas. C. Hart Seed Co., Wethersfield, Connecticut) for INSV transmission. The leaves were changed every day until the thrips died. Leaves with virus symptoms were then used as the source of inoculum to transmit INSV to other thrips.

Effect of Nematode Parasitization on INSV Infection and Propagation in Thrips

Exp. 1. INSV detection rates in thrips exposed to nematode parasitization versus control thrips not exposed to nematodes ('Virus and nematode-together' experiment). Four nematode-parasitized adult female thrips (the source of nematodes) were released into a thrips rearing box containing 50 newborn larval thrips on three impatiens leaves with INSV symptoms (the source of INSV) rooted in a cotton pad. After incubation of the leaves for nine days at 24 °C, I collected newly emerged adult thrips daily. Because virus transmission ability is lower in the first one or two days after emergence of the adult (Wijkamp et al., 1996), the adult thrips were kept alive on a leaf disk on water-soaked cotton in a diet cup for three days. Each thrips was, then, chopped up with sharp tweezers in 110 µl extract buffer (0059, Agdia) on a microscope slide glass under a dissecting microscope. The nematode parasitization of each thrips was confirmed in this process, and the thrips extract was tested in ELISA to detect the virus. This procedure was replicated ten times for the treatment and the control, i.e., thrips without contact with nematodes but fed on INSV-infected impatiens leaves. The percentage of INSV-positive thrips was obtained for both the treatment and the control. Separately, I also calculated the percentage of INSV-positive thrips for the nematode-parasitized and for the non-parasitized thrips in the nematode-treatment group to compare with detection rate of the control group. Percentage data were arcsine transformed and analyzed with the *t*-test to find any significant differences in INSV detection rate. In addition, the frequencies of thrips with (1) both nematode and INSV, (2) with nematode and without INSV, (3) without

nematode and with INSV, and (4) without either nematode or INSV were counted to build a contingency table in each replication of nematode treatment. After the heterogeneity analysis had been performed, the contingency tables were pooled and analyzed by Chi-square with Yates correction (Zar, 1996) to identify any significant difference in INSV detection between thrips with nematode parasitization and without nematode parasitization.

To identify mechanisms explaining the results of this experiment, i.e., a difference in INSV detection rate between thrips with and without nematode parasitization, I conducted three subsequent INSV experiments and one feeding experiment, testing each of four hypotheses. H_1: nematodes prefer to penetrate healthy thrips rather than INSV-infected thrips; H_2: parasitized first instar thrips are less able to acquire virus during feedings; H_3: INSV propagation is suppressed in parasitized thrips; or H_4: parasitized larvae thrips have less opportunity to contact the virus because of lower feeding activity.

Exp. 2. Preference of nematodes for INSV-infected thrips versus healthy thrips ('Virus-first' experiment). Choice tests were conducted by offering INSV-acquired and healthy thrips to nematodes, as follows. An INSV-acquired thrips was prepared by feeding a newborn larva with an impatiens leaf with INSV symptoms for five days. The potentially INSV-acquired larva thrips was then paired with a five-day old larval thrips that had only fed on a healthy leaf, and both thrips were exposed to one nematode-parasitized adult female thrips in a pipette-tip arena (Fig. 2). One day after the incubation, nematode parasitization and the number of nematodes penetrated

the host were confirmed by dissecting each kind of thrips. If there was no nematode parasitization in either thrips, I did not test them for INSV infection and dropped them from the analysis. For pairs of thrips in which at least one thrips was parasitized by the nematode, samples were prepared for ELISA in the same manner as described above (Exp. 1). This choice test was run for 36 pairs. I analyzed the mean number of nematodes that penetrated thrips using a paired *t*-test to determine if nematodes showed any preference for INSV-infected or healthy thrips.

Exp. 3. Difference in INSV acquisition between thrips with and without nematode parasitization ('Nematode-first' experiment). A newborn larva was transferred to a pipette arena and exposed to parasitized females for 12 h. This short exposure increased the period remaining during which the larva could still successfully acquire virus (Wetering et al., 1996). Thrips for the control group were prepared in same way, except they were not exposed to a nematode-parasitized adult female thrips. Each thrips from the treatment groups was then paired to a control thrips and transferred to a plastic diet cup with an impatiens leaf with INSV symptoms rooted in cotton. After four days of incubation at 24 °C, both the nematode-exposed and control thrips were then dissected to detect nematode parasitization and prepare them for ELISA to detect INSV, following the same method as described above. If the nematode-exposed thrips in a pair was found not to be parasitized, I discarded the pair. These procedures were repeated until I got 30 pairs for analysis with Fisher's exact test of contingency tables (Zar, 1996).

Exp. 4. Effect of nematode presence on INSV propagation and transmission by thrips. Groups of 25 newborn larval thrips were fed on an impatiens leaf (one group per leaf) with INSV symptoms for four days at 24 °C in a growth chamber to permit virus acquisition. Half of the surviving thrips (mostly second instars) were then exposed as a group to two nematode-parasitized adult female thrips for a day in a pipette-tip arena. The remaining thrips were placed in a pipette-tip arena, but without any nematode-parasitized thrips. Thrips from each group were then transferred to individual diet cups containing bean leaf disks on soaked cotton and held in the growth chamber for 0, 5 or 10 days before testing for the presence of INSV.

On day 0 (the same day as the nematode exposure), a thrips was randomly chosen from the nematode-exposed group and chopped up in 110 µl extract buffer on a microscope slide glass for identification of nematode parasitization. If parasitization was detected, I tested the thrips extract to detect INSV by ELISA. If I did not detect nematode parasitization in the thrips, I chose another thrips from the nematode-exposed group and also dissected it for nematode detection. This procedure was repeated until a nematode-parasitized thrips was located from the nematode-exposed group. Next, also on day 0, I randomly chose a thrips from the control group and processed as described above to confirm the absence of nematodes and to test with ELISA. These two thrips were considered a pair and I repeated the procedure until 30 pairs had been tested. The numbers of parasitized and non-parasitized thrips testing positive for INSV were recorded to construct a contingency table for analysis with Fisher's exact test (Zar, 1996).

On day 5, using the same protocol described above, another 30 pairs were prepared from the two groups of thrips. The same statistical analysis was followed after confirmation of nematode parasitization and INSV infection and construction of a contingency table.

The remaining thrips in both groups were used for two different experiments—one for the day 10 assessment of the effect of parasitization on INSV propagation and, the second, on the difference in INSV transmission rates between viruliferous adult thrips with and without nematode parasitization, using an impatiens leaf assay. I obtained data for both of these events (effect on INSV propagation and effect on INSV transmission) from the same groups of thrips, using a series of steps, as follows. On day 9 (after the exposure to nematodes) impatiens leaves in all diet cups (both groups of thrips) were individually replaced with fresh impatiens leaves to assess the INSV transmission ability of the adult thrips with different parasitism histories. On day 10 (after an inoculation feeding period of 1 day), I randomly chose a diet cup containing a thrips that had been exposed to nematodes and processed and tested with ELISA as described. If I found nematode parasitization in the thrips, I also collected the leaf from the diet cup and held it at 24 °C in a growth chamber to check for development of virus symptoms. If symptoms were found in a leaf, then 3 days later I ground the leaf in 330 µl of extract buffer, and the sap was tested with ELISA for INSV infection. All other leaves (without visible INSV symptom) were also processed for ELISA ten days later because INSV infected leaves do not always show symptoms. I conducted the same procedure for a randomly chosen thrips from the control group (never exposed to nematodes). But, if the sex of the thrips from the control group was

different from that of treatment group, I discard the control thrips and chose another thrips randomly from the rest of the group until I found a control thrips of the same sex. This procedure was adopted because there are differential transmission rates of tospoviruses between adult female and male thrips (Sakurai et al., 1998; Wetering et al., 1998). Leaves fed on by control thrips were also processed as described above. These procedures were repeated until I obtained 30 pairs of adult thrips (one of each pair with and one without nematode parasitization) and 30 pairs of leaves fed on by test thrips. From these data, I constructed one contingency table for 30 thrips pairs (± nematode parasitization and ± INSV) and two contingency tables from the 30 leaf pairs, one for the frequency of leaves with INSV symptoms on leaves and the other for the frequency of leaves testing positive for INSV in ELISA. These three contingency tables were analyzed by Fisher's exact test to find any significance differences (Zar, 1996).

Effect of Nematode Parasitization on Feeding Behavior of Thrips

Exp. 5. Leaf. Larvae that were less than 6 h-old were parasitized by exposing them to a nematode-parasitized thrips for one day in a pipette-tip arena. Healthy first instars were also held under the same physical conditions (pipette-tip arena) except for lack of exposure to the nematode-parasitized thrips. Individual thrips were then transferred to leaf disks on water-soaked cotton in diet cups and held in a growth chamber at 24 °C. Leaf disks were prepared with a paper punch which make approx. 33.2 mm^2 area. Leaf disks were replaced daily and fed-on disks were photographed

under a dissecting microscope (100×) using a digital camera (DP11, Olympus, Japan). The damaged area on the leaf disk was measured using SigmascanPro (SPSS Science, Chicago, Illinois). Daily measurement of leaf damage was continued until the thrips became a prepupa, a non-feeding stage, and then the presence of a nematode (in thrips exposed to nematodes) was confirmed by dissecting the prepupa under the microscope. The sex of the prepupa was also determined. Differences in daily mean leaf damage and total leaf damage (from first instar through second instar) between parasitized and healthy thrips were analyzed with a *t*-test. In addition, to measure feeding damage on the leaf disk by adult thrips, I collected female pupae (of which parasitization status was unknown) from the nematode rearing cages and placed them individually in diet cups with a same leaf disk describe above. After adult emergence, the leaf disks were replaced daily and fed-on disks were processed same as described above.

Exp. 6. Honey. An adult female thrips (of which parasitization status was unknown) was collected from the nematode rearing box and transferred into the pipette-tip arena with only a drop of distilled water. After incubation for 1 d at 24 °C in a growth chamber, the thrips was placed in an arena between two glass microscope slides of the same design as used to confirm nematode parasitization in the nematode rearing method. Pure honey was placed on the inside surface of one of the glass slides and I directly observed the thrips' feeding behavior. I measured duration and frequency of feeding and resting bouts by observing the bottom of the slide where the honey drop was placed for 1 h. Frequency of feeding was counted as the number of probing (stylet penetration) events. Presence of nematode parasitization was

confirmed by dissecting the thrips under the microscope after the observation period. This procedure replicated until I tested 17 parasitized and 17 healthy thrips. The duration and frequency of feeding and resting were analyzed with a *t*-test.

Exp. 7. Pollen. A pupal thrips (of which parasitization status was unknown) was collected from the nematode rearing box and placed in the pipette tip arena, with a drop of distilled water in a growth chamber at 24 °C. After an adult emerged, it was held in the arena for one more day with only distilled water. The next day, I placed the thrips in a glass-slide arena with castor bean pollen grains for 1 h. The pollen grains were placed on the inside surface of one of the slides with a paintbrush. The number of pollen grains that were fed on by the thrips was counted by observing bottom of the slide where the pollen grains were placed under a compound microscope. If the surfaces of the glass slides were dry, pollen grains were wrinkled and could not be distinguished from fed-on pollen grains. However, by adding a drop of distilled water, the wrinkled pollen grains expanded and fed-on pollen grains could be distinguished by the collapsed outer wall. The number of fed-on pollen grains for adult females (13 parasitized and 25 healthy) and adult males (25 parasitized and 38 healthy) were analyzed with two-way ANOVA using GLM procedure to identify significant effects of sex and parasitization (SAS Institute, 1995).

Results

Effect of Nematode Parasitization on INSV Infection and Propagation in Thrips

Exp. 1. INSV detection rates in thrips exposed to nematode parasitization versus control thrips not exposed to nematodes ('Virus and nematode-together' experiment). When thrips larvae were exposed to INSV inoculum and nematodes simultaneously, the mean INSV detection rate of the nematode-exposed group as a whole was 22.2%, approximately 1/3 lower than the INSV detection rate for the control group (32.1%) (Table 3). However, the difference was not statistically significant ($t = 1.48$, d.f. = 18, P = 0.156). When I compared INSV detection rates of subgroups in the nematode-exposed group with the INSV detection rate in the controls, a significant difference was found for the subgroup of thrips that were actually parasitized ($t = 2.64$, d.f. = 18, P = 0.017), but this was not the case for the subgroup that were not parasitized ($t = 2.64$, d.f. = 18, P = 0.388) (Table 3). When all thrips in the experiment were classified into a 2 × 2 table for nematode parasitism and INSV infection, I found a negative relationship between nematode parasitism and INSV infection (heterogeneity analysis $\chi^2 = 11.01$, d.f. = 8, P = 0.210; Chi-square test with Yates correction $\chi^2 = 4.71$, d.f. = 1, P = 0.032) in the pooled contingency table. Overall in the pooled data, only 16.7% of the parasitized thrips were INSV-positive, versus 28.7% of the healthy thrips.

Exp. 2. Preference of nematodes for the INSV-infected thrips versus healthy thrips ('Virus-first' experiment). Nematodes were found to attack thrips with and without INSV infection at equal rates. The mean numbers of nematodes per thrips that attacked INSV-infected and healthy thrips were 1.9 and 2.0, respectively. Both thrips were attacked in 20 pairs and only one thrips in the remaining 16 pairs. Of the latter parasitized thrips, eight were INSV positive and eight negative. There was no statistical difference between attack rates in healthy versus INSV-infected thrips (t = 0.25, d.f. = 35, P = 0.807).

Exp. 3. Difference in INSV acquisition between thrips with and without nematode parasitization ('Nematode-first' experiment). Of 30 pairs of thrips (one healthy and one parasitized by nematodes) exposed to INSV, in four pairs both thrips was positive for INSV, in three pairs only parasitized thrips did so, and in four pairs only the healthy thrips was positive for INSV. There was no difference in INSV detection rate between thrips with and without nematode parasitization (P = 0.776).

Exp. 4. Effect of nematode presence on INSV propagation and transmission by thrips. The rate of INSV detection, in both thrips with and without nematode parasitization, increased as thrips aged after exposure to an impatiens leaf with INSV symptom for four days. The difference in INSV detection in thrips with and without nematode parasitization was highest ten days after parasitization, but this difference was not statistically significant (P = 0.066, Table 4). The thrips tested ten days after nematode parasitization consisted of 18 female and 12 male pairs. Results

of INSV transmission to new impatiens leaves showed no difference in transmission rate between the 16 nematode-parasitized thrips and 23 non-parasitized thrips (Table 5). All impatiens leaves with INSV symptoms as well as thrips that transmitted INSV to the impatiens leaves (of which INSV infection was confirmed by symptom development or ELISA) were all found to be positive in the ELISA.

Effect of Nematode Parasitization on Feeding Behavior of Thrips

Exp. 5. Leaf. In larval thrips, there was no significant effect of nematode parasitization on either daily mean ($t = 1.09$, d.f. = 44, $P = 0.280$) or total feeding damage ($t = 0.66$, d.f. = 44, $P = 0.514$) during the two larval instars (Table 6). However, for adult female thrips daily mean feeding damage was reduced by 81% in thrips with nematode parasitization versus healthy thrips ($t = 6.02$, 48, d.f. = 52, $P < 0.001$).

Exp. 6. Honey. Honey feeding decreased by 22% in nematode-parasitized adult female thrips compared to healthy adult female thrips ($t = 2.17$, d.f. = 32, $P = 0.038$), but feeding frequency was not affected by the nematode parasitization ($t = 0.33$, d.f. = 32, $P = 0.746$). Resting time increased by 33% in nematode-parasitized adult female thrips, but this change was not statistically significant ($t = 1.63$, d.f. = 32, $P = 0.114$). Nematode parasitization did not affect resting frequency ($t = 0.06$, d.f. = 32, $P = 0.952$) (Table 7).

Exp. 7. Pollen. The number of pollen grains fed-on was reduced by 38% in nematode-parasitized adult female thrips and 21% in nematode-parasitized male thrips compared to non-parasitized adult female and male thrips, respectively. Differences in the number of pollen grains fed-on by female and male thrips were both statistically ignificant (two-way ANOVA; sex $F_{1, 97} = 41.09$, $P < 0.001$; nematode parasitization $F_{1, 97} = 14.06$, $P < 0.001$; interaction $F_{1, 97} = 4.98$, $P = 0.028$) (Table 8).

Discussion

In my initial experiment (Exp. 1), I showed that nematode parasitization was associated with a lower rate of INSV detection in *F. occidentalis*. As possible mechanisms responsible for this result, I proposed and tested four hypotheses. Theoretically, the decreased INSV detection rate among nematode-parasitized thrips might have been due to (1) a preference of nematodes to penetrate healthy thrips more often than INSV-infected thrips; (2) a lower virus acquisition rate by parasitized first instar thrips; (3) a depression of INSV propagation in parasitized thrips; or (4) a lower rate of feeding by parasitized larvae thrips, leading to less opportunity to contact the virus. Because Exp. 2 showed there was no difference in attack rate by nematodes between healthy and INSV-infected thrips, and no difference in the average number of nematodes inside attacked thrips of these categories, hypothesis 1 was falsified. The results of the nematode-first experiment (Exp. 3) show that nematode parasitization has no effect on virus acquisition by first instar thrips. However this may not provide enough evidence to reject the hypothesis 2, because the nematode parasitization might

decrease feeding behavior of the host so as to reduce opportunities to pick up the virus over the course of the life span of the larvae stages of the thrips. However, in Exp. 5 (Table 6), I found no statistical difference in total leaf damage caused by larval thrips between the nematode-parasitized and non-parasitized individuals, causing us to reject hypothesis 4. Interestingly, in the same experiments with adult thrips, leaf damage was significantly lower for parasitized thrips than healthy thrips (Table 6). This same reduction was also found for honey feeding (Table 7) and pollen feeding (Table 8). These results support a previous finding that the nematode parasitization does not reduce survivorship of larval thrips (Fig. 5), but does harm adults (Table 1).

Experiment 4 (Table 4) on effect of nematode presence on virus propagation inside thrips seems to provide the best-supported explanation for the decrease in INSV detection in parasitized thrips. Even though no significant differences were detected, there was a consistently lower INSV detection rate in nematode-parasitized thrips.

The similar rate of INSV transmission by parasitized and healthy thrips (Table 5) can perhaps be explained by the results of the feeding experiment (Table 7). Although feeding time of parasitized thrips eating honey was significantly shorter than that of healthy thrips, their probing frequency was not different. Because virus inoculation by tospovirus-infected thrips is believed to occur during stylet penetration and salivation (rather than food uptake) (Nagata and Peters, 2001), the finding that lowered feeding does not result in lowered transmission may be explained by a failure of the probing rate to decline. This assumes that the probing rate in honey would be reflective of that in leaf tissue. In spite of reduced feeding activity in parasitized thrips, lack of difference in probing frequency may represent a difficulty in feeding as

reported for other parasitized insects (Killick-Kendrick et al., 1977; Jenni et al., 1980) tending to probe more frequently to combat the nutritional deficit due to parasitism and taking longer to engorge.

In conclusion, reduced INSV detection was found among thrips population with nematode parasitism due to negative effects of the parasitization on virus propagation in thrips, but there was no difference in INSV transmission ability between viruliferous parasitized and non-parasitized thrips. The feeding activity was significantly reduced by the nematode parasitization in adult thrips, but not in larvae. These studies suggest that nematode-parasitized adult thrips could be released for thrips control without increasing feeding damage to the crop, and INSV acquisition and transmission would be less likely.

CHAPTER III

POPULATION DYNAMICS OF NEMATODE TRANSMISSION

Introduction

Thripinema nicklewoodi Siddiqi (Tylenchida: Allantonematidae), first recorded as a parasite of two blueberry thrips in Canada (Nickle and Wood, 1964), also attacks *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) (Wilson and Cooley, 1972; Greene and Parrella, 1993), a major agricultural pest worldwide (Robb, 1989; Shipp and Zariffa, 1991; Higgins, 1992).

The nematode attacks any post-egg life stage of *F. occidentalis* (Table 2; Mason and Heinz, 2002). But, in a laboratory choice-test, transmission occurred most often from adults to younger life stages (first instar, second instar, and prepupa) (Table 2). The nematode neither kills the larvae hosts (Fig. 5) nor affects their feeding behavior (Table 6). In the adult stage of the host, however, nematodes have deleterious effects on reproduction (Nickle and Wood, 1964; Wilson and Cooley, 1972), survivorship (with a greater effect on males than females) (Table 1), and feeding behavior (Tables 6, 7, and 8). Despite the harmlessness of parasitization to larval thrips, *T. nicklewoodi* may still have potential as a biological control agent of *F. occidentalis*, as proposed earlier (Greene and Parrella, 1993; Heinz *et al.*, 1996; Loomans *et al.*, 1997; Arthurs and Heinz, 2002). Because the nematode suppresses feeding activity and shortens adult lifespan, transmission of tospoviruses, one of major

concern in managing *F. occidentalis*, may be less likely in thrips population with the nematode than thrips population without the nematode (Chapter II). However, there have been few studies of the population dynamics of *T. nicklewoodi* and *F. occidentalis* to determine if nematode release can lead to thrips population suppression. Population dynamics or seasonal abundance of *Thripinema* nematodes have been studied in *Aptinothrips rufus* Gmelin (Sharga, 1932; Lysaght, 1937), *Megaluriothrips* sp. (Reddy *et al.*, 1982), *F. occidentalis* (Heinz *et al*, 1996), *F. fusca* Hinds (Funderburk *et al.*, 2002a), and *F. australis* Morgan (Funderburk *et al.*, 2002b).

My aim in this study was to determine how nematode parasitism affected the dynamics of *F. occidentalis* populations by observing trends of two experimental thrips populations with different initial parasitism rates. Objectives were (1) to determine if nematode transmission could persist in experimental host populations under semi-field conditions, on caged impatiens plants; (2) to assess the effects of the nematode transmission on the population dynamics of four major thrips life stages (1st instar, 2nd instar, adult female, and adult male), and (3) to assess the effect of differential survivorship of parasitized female and male adult thrips on the dynamics of sex ratio in thrips populations. The results of this study provide information on the potential for managing *F. occidentalis* using *T. nicklewoodi* as a biological control agent.

Materials and Methods

Experimental Design

Thirty wooden frame cages (33 W × 60 L × 33 H cm) were constructed and covered with fine-mesh bags made of two materials—a transparent Nitex (Sefar America INC, Kansas City, MO) for the upper surface (which allowed us to count number of flowers without opening the cages) and a semi-transparent Sunline (Sankei, Japan) fabric for the other parts of the bag. All the cages were placed in a plastic hoop greenhouse (5.2 W × 3.7 L × 2.4 H m), and nine individually potted impatiens plants (*Impatiens walleriana* Hook. f., var. 'Super Elfin White') were placed in 7.6 × 7.6 cm pots in each cage. Extra potted impatiens plants were kept in large wooden frame cages (60 W × 115 L × 33 H cm) covered with bags in the greenhouse for later addition to experimental cages as needed to support expanding thrips populations (One new plant was added when any given cage had fewer than 10 flowers total in daily counts, Table 9). Before the experiment began, all test cages, stocked with plants, were held in the same greenhouse from March 17, 2002 to April 1, 2002 to await flowering. Because impatiens plugs were purchased from a commercial plug grower (in Northfield, MA), I sampled half of the flowers in each cage before starting the experiment to look for any pre-existing thrips. Plants in three cages (3/30, 10%) had thrips (two cages had one larvae thrips each and one cage had one adult thrips). Therefore, for balance one of each of these three pre-infested cages was randomly assigned to each of the three treatments, i.e., 'low nematode,' 'high nematode,' and

control, respectively. The remaining 27 cages were assigned at random to the treatments. On the next day (April 2, 2002), the flowers in each cage were counted, and the averages per treatment were 3.0 (± 0.2 SD), 3.3 (± 0.3 SD), and 3.3 (± 0.2 SD) for 'low nematode', 'high nematode' treatment, and control, respectively, which were not statistically different in ANOVA ($F_{2, 29} = 0.45$, $P = 0.645$) (Table 10). On the same day, I released three healthy female and three healthy male thrips in each cage. Six, 8, and 10 days after the release of the healthy thrips, parasitized female thrips were released into the 'low nematode' and 'high nematode' treatment cages. A total of three parasitized thrips were released per cage in the 'low nematode' treatment (creating a level of 50% parasitism among adult females) and seven parasitized adult female thrips were placed in each cage of the 'high nematode' treatment (yielding an initial level of 70% adult female parasitism). In 'low nematode' treatment cages, one parasitized thrips was released on each release day but, in the 'high nematode' treatment cages, two, two, and three parasitized thrips were released 6, 8, and 10 days after the release of the healthy thrips. Parasitized thrips were released after healthy thrips to ensure prior reproduction of the healthy thrips, so that nematode transmission could occur among the resulting immature thrips of next generation. Releases of parasitized thrips were distributed over three release dates to promote better host and parasite synchrony.

Sampling Methods and Statistical Analysis

Because population trends of adult *F. occidentalis* based on counts of thrips in blossoms have the best correlation with whole plant counts (Shipp and Zariffa, 1991), I sampled impatiens flowers to estimate total thrips (in the flower niche) in each cage on each sample day (Table 10). Samples were collected every 200 degree days (DD), following the first release of healthy adult thrips. Two hundred DD was selected as the between-sample interval by adding 26.5 DD (to provide time for mating and egg development) to 173.5 DD (the time needed for development from egg to adult), as estimated by Robb (1989), assuming a 10 °C lower threshold and 34 °C upper threshold at fluctuating temperatures. Temperatures were recorded using three Hobo H8 data loggers (Onset Computer Corporation, Bourne, MA) placed inside of three randomly selected cages in the greenhouse. Daily 24 h mean temperatures were calculated from each instrument and averaged to obtain the daily mean temperature values used to calculate the number of DD per day using the 10 °C lower limit.

Over the whole experiment, I took samples seven times. On each date, I first counted the total number of flowers in each cage. I then picked one third of the flowers, and dipped these flowers in a 0.03% dishwashing detergent (Sun & Earth, Inc, Norristown, Pennsylvania) solution in a 148 ml plastic container. The treatment and cage numbers of all samples were marked on the lid of each container and all containers were taken to the laboratory for counting and identification of the thrips. Petals and sepals detached from the flowers in the detergent solution. Floating flower parts were removed one by one using tweezers, swirling them first to leave thrips

behind. The containers were then held for 5 min to let thrips sink. Thrips on the bottom of each container were then removed with a micropipette under a dissecting microscope and placed on a glass slide for counting and identification (Mound and Kibby, 1998). Nematode parasitization of thrips was confirmed by dissecting the thrips. I found this method to be more efficient than washing and sieving (or filtering). Because all adults thrips recovered during the whole experimental period were *F. occidentalis*, I assumed all immature thrips that I collected were also *F. occidentalis*. I made this assumption because immature thrips can not be as readily identified to species as adults.

The number of healthy and parasitized thrips per cage was obtained by multiplying the number of thrips per flower within a cage by the number of flowers in the cage. This was done separately for each of four subgroups, i.e., 1st instar, 2nd instar, adult female, and adult male. These data were analyzed by ANOVA using GLM procedure of SAS (SAS Institute, 1995) to identify significant effects of different levels of nematode introduction on densities of healthy thrips populations. If significance in ANOVA was detected, data were further analyzed using Tukey's studentized range honestly significant difference (HSD) test for multiple comparisons (SAS Institute, 1995).

Because I never found any parasitized thrips in control cages, the numbers of parasitized thrips in the 'low nematode' and 'high nematode' treatments were compared using a *t* test. Percent parasitism rates in the 'low nematode' and 'high nematode' treatments were also calculated and compared using a *t* test, after arcsine transformation (Zar, 1996). In addition, I conducted the Kruskal-Wallis single factor

analysis of variance by rank to detect differences in percent parasitism among thrips subpopulation in each treatment (Zar, 1996). If significance in the Kruskal-Wallis test was detected, multiple comparisons were conducted using the Dunn test (Zar, 1996).

Sex ratios (proportion male) of healthy thrips were obtained in each cage by dividing the number of adult males by the sum of adult males and females. The sex ratios among the two treatments and control were analyzed by ANOVA using GLM procedure of SAS (SAS Institute, 1995) after arcsine transformation (Zar, 1996) to identify significant effects of different level of nematode introduction on sex ratio of the healthy thrips population. Any significant difference between treatments was identified by Tukey's studentized range HSD test after the ANOVA. In addition, I compared the sex ratios of parasitized thrips to that of the healthy thrips, pairing data by cage. Because of the absence of independence in the two sex ratios in the same cage, I compared the sex ratio between healthy and parasitized thrips using paired *t* test.

Results

Effect of Nematode on Healthy Thrips Subpopulations

Trends in thrips density by life stage and treatment are given in Fig. 11. Compared to control cages, significant reductions in healthy thrips numbers were found only for adult females (on 800 DD: $F_{2, 29} = 8.26$, $P = 0.002$; on 1000 DD: $F_{2, 29} = 9.35$, $P = 0.001$; on 1200 DD: $F_{2, 29} = 16.22$, $P < 0.001$) and for adult males (on 400

DD: $F_{2, 29} = 13.17$, $P < 0.001$), for both 'low nematode' and 'high nematode' treatments (Fig. 11). There were also significant reductions in healthy adult thrips in only the 'low nematode' treatment on 1400 DD (adult females: $F_{2, 29} = 3.59$, $P = 0.042$) and on 1200 DD (adult males: $F_{2, 29} = 3.26$, $P = 0.054$) (Fig. 11). Percent reductions in number of adult female thrips were 47, 50, 56, 79, and 59% in 'low nematode' and 27, 63, 69, 73, and 39% in 'high nematode' on 600, 800, 1000, 1200, 1400 DD, respectively. No differences, however, were found between 'low nematode' and 'high nematode' treatments on any DD of any life stages from Tukey's studentized range HSD test.

Persistence of Parasitism by Treatments

No parasitized thrips were ever found in control cages. A significant difference in number of parasitized thrips between the two nematode treatments was found only for 2nd instars in the 1200 DD sample, in which more parasitized individuals were found in the 'low nematode' treatment than the 'high nematode' ($t = 2.58$, d.f. = 18, $P = 0.019$) (Fig. 12). When I analyzed rates of parasitism after arcsine transformation, no significant differences were found between the two nematodes release treatments on any sampling DD of any life stages. There was convergence in both number of parasitized thrips (Fig. 12) and percent parasitism among all the subpopulations of *F. occidentalis* investigated (Fig. 13).

Parasitism among Thrips Subpopulations

In both 'low nematode' and 'high nematode' treatments, parasitism in adult females was always higher than in other life stages, except on 1400 DD. Statistical difference was found in samples on 400 DD ($\chi^2 = 12.55$, d.f. = 3, $P = 0.006$), 600 DD ($\chi^2 = 10.09$, d.f. = 3, $P = 0.018$), 1000 DD ($\chi^2 = 11.63$, d.f. = 3, $P = 0.009$), and 1200 DD ($\chi^2 = 7.64$, d.f. = 3, $P = 0.054$) in the 'low nematode' treatment and on 600 DD ($\chi^2 = 16.69$, d.f. = 3, $P = 0.001$) and 1000 DD ($\chi^2 = 9.05$, d.f. = 3, $P = 0.029$) in the 'high nematode' treatment (Fig. 13) in Kruskal-Wallis tests. Data for adult female and male thrips on 200 DD were neither analyzed nor plotted in the Fig. 13 (and also omitted in Figs. 14 and 15) because only two observations was made in both 'low nematode' and 'high nematode' treatment.

Effect of Nematode Parasitism on Host Sex-ratio

Sex ratios of healthy thrips in populations with nematode parasitism were more male-biased than those of control populations without nematodes for all samples after 600 DD (Fig. 14). Statistical difference, however, was found only between 'high nematode' treatment and control on 800 DD ($F_{2, 28} = 3.78$, $P = 0.036$). When I compared sex ratios of healthy and parasitized thrips in both 'low nematode' and 'high nematode' treatments, sex ratio was always higher (more male biased) in healthy thrips than in parasitized thrips, except in samples on 400 and 1400 DD of the 'low nematode' treatment. Statistical differences were found on 600 DD ($t = 2.37$, d.f. = 8,

$P = 0.045$), 800 DD ($t = 3.83$, d.f. = 8, $P = 0.005$), and 1000 DD ($t = 2.54$, d.f. = 8, $P = 0.035$) in the 'low nematode' treatment and on 600 DD ($t = 2.55$, d.f. = 7, $P = 0.038$), 800 DD ($t = 5.00$, d.f. = 7, $P = 0.002$), 1000 DD ($t = 3.31$, d.f. = 8, $P = 0.011$), and 1200 DD ($t = 2.61$, d.f. = 6, $P = 0.040$) in the 'high nematode' treatment (Fig. 15).

Discussion

Persistence in parasitism for seven host generations was observed in both the 'low nematode' and the 'high nematode' treatments despite differences in the initial parasitism rate. The percent parasitism of adult female thrips converged to around 30% after seven generations. The lack of difference in parasitism levels in the two populations might have been caused by saturation of available hosts at high parasite densities (Knell *et al.*, 1996). This would occur if at times all available immature thrips in flowers were to become infected. If this happens, the *per capita* transmission would decline. This may have been the case in the 'high nematode' treatment in the beginning of the experiment, when thrips numbers were very low. Given the facts that larvae stages of *F. occidentalis* are more abundant on leaves than flowers, and adult thrips aggregate in flowers (Higgins, 1992; Reitz, 2002), it is possible that at times new hosts were unavailable to nematodes.

Significant reduction in *F. occidentalis* density due to *T. nicklewoodi* parasitization was found, but only in the adult stage (Fig. 11). Even with up to 50% parasitism of adult females in both the 'low nematode' and 'high nematode' treatments (Fig. 13), the density of larvae thrips in the next generation was not reduced. This lack

of effect on larvae might be explained if the *per capita* reproductive rates of healthy adult female thrips in the nematode treatments were to increase due to reduced competition. As shown in Tables 1 and 2, the number of flowers in control cages was always lower than in the other two treatments, except on the first two sampling dates even though extra impatiens plants were added whenever there were fewer than ten flowers in any given cage. This inference may explain why results from my experiment are in contrast to those of a two-year field study by Funderburk *et al.* (2002a) who concluded that larval numbers of *F. fusca* collected from flowers and terminal buds of peanut declined in late season as parasitism of adult female thrips by *T. fuscum* increased in mid season.

Parasitism rates in adult male thrips were lower than in adult female thrips (Fig. 13), in contrast to a previous finding (Fig. 4). Differential survivorship between parasitized adult female and male thrips might be an underlying mechanism for the discrepancy. Because nematode parasitization causes death earlier in male thrips than in female thrips (Table 1), there might be less chance for parasitized males to be sampled. This is also supported by data in Fig. 15 that show that sex ratio was significantly lower among parasitized thrips than among healthy thrips. Such early death would also provide explanations for previous findings of lower parasitism in males (Funderburk *et al.*, 2002b) or its complete absence (Sharga, 1932) in adult male compared to adult female thrips associated with *Thripinema* sp. nematodes. In spite of the equal chance for adult female and male thrips to be parasitized by the nematode and the more deleterious effect on adult male thrips by *T. nicklewoodi* compared to adult females (Fig. 4 and Table 1), the number of adult male thrips in both treatments

was the same as that in the control population. This result is contrary to my prediction, which was for a similar degree of decline in adult female and male thrips (or even more reduction in adult males).

The apparent lower parasitism rates in larval thrips (Fig. 13) might be an artifact caused by the reduced probability of parasitization due to the shorter lifespan of larvae compared to adults. This may be the reason why, in previous studies, none (Wilson and Cooley, 1972) or few (Lysaght, 1937) of immature thrips were found to be parasitized by the nematode in field samples.

In conclusion, *T. nickelwoodi* applied to experimental populations of *F. occidentalis* persisted for seven generations and reduced the density of adult female thrips by up to 79% on caged impatiens in greenhouse. The nematodes, however, did not suppress the other life stages of *F. occidentalis* investigated, 1st, 2nd instar larvae, and adult male thrips.

CHAPTER IV

NEMATODE REARING AND RELEASE FOR THRIPS CONTROL

Introduction

Frankliniella occidentalis Pergande (Thysanoptera: Thripidae), injures many field and greenhouse crops worldwide, both by transmitting tospoviruses and by direct feeding (Yudin *et al.*, 1986; Robb, 1989; Daughtrey *et al.*, 1997; Jacobson, 1997; Lewis, 1997). Over the last decade, *F. occidentalis* has become widespread in greenhouses, causing severe damage to food and ornamental crops (Jacobson, 1997; Lewis, 1997). The high capital investment and production costs associated with greenhouse crops increase financial losses from thrips damage (Lewis, 1997).

Various groups of natural enemies have been investigated for the control of thrips: phytoseiid mites, anthocorid bugs, hymenopterous parasitoids, fungal pathogens, predatory thrips, and nematodes (Lewis, 1997). The latter three groups have not been studied enough to allow commercial applications, although the potential of entomopathogenic nematode such as species in the genera *Steinernema* and *Heterorhabditis* has been evaluated for control of thrips in soil and compost (Helyer *et al.*, 1995; Ebssa *et al.*, 2001). Currently, phytoseiid mites in the genus of *Amblyseius* and anthocorids in the genus of *Orius* are used for the biological control of thrips in different crops (Tellier and Steiner, 1990; van Houten *et al.*, 1995; Frescata and Mexia, 1996; Funderburk *et al.*, 2000; Jacobson *et al.*, 2001). Among *Amblyseius* species, *A.*

cucumeris Oudemans (Acari: Phytoseiidae) became the favored species for the control of thrips and has been used extensively in programs of biological control in protected edible crops such as sweet pepper and cucumber (Jacobson *et al.*, 2001).

Recently, a nematode in the genus of *Thripinema* (Tylenchida: Allantonematidae) has been proposed as a potential biological control agent of thrips (Greene and Parrella, 1993; Heinz *et al.*, 1996; Arthurs and Heinz, 2002; Mason and Heinz, 2002; Funderburk *et al.*, 2002ab). *Thripinema nicklewoodi* Siddiqi, obligate parasite of *F. occidentalis*, can parasitize any post-egg life stages (Table 2; Mason and Heinz, 2002) of the thrips and transmission is believed to occur in the plant canopy (Greene and Parrella, 1993). *T. nicklewoodi* caused 19-33% parasitism in adult female *F. occidentalis* collected from alfalfa and roses in several areas of California (Greene and Parrella, 1993) and, in the previous study (in Chapter III) on caged impatiens, nematode reduced populations of adult female *F. occidentalis* by 50-79%, although there were no reduction in larval numbers (Fig. 11).

The production of flowering plants in greenhouses is among the fastest growing sectors of agriculture (Held *et al.*, 2001). However, ornamental crops have been hindered from adopting biological control because of the short production cycle, the very low pest infestation levels tolerated by growers and consumers, and the diversity of both crop types and growing conditions (De Courcy Williams, 2001). Hence, most growers rely on insecticides to manage arthropod pests, but this option is increasingly restricted because of environmental, worker, and consumer safety issues (Held *et al.*, 2001). Biological control using *T. nicklewoodi* may be an alternative method, because parasitization causes sterilization in adult thrips (Nickle and Wood,

1964; Greene and Parrella, 1993), reduced feeding activity in adult stages, and does not increase the spread of tospoviruses that is common negative side effect of insecticide application (Chapter II). However, the absence of economic *in vitro* rearing methods currently hinders the nematode from inundative or augmentative release to control thrips. Although a previously developed *in vivo* rearing method (Arthurs and Heinz, 2002) can double numbers of parasitized thrips used as inoculum, this method is not economical for mass production because it has too many steps in each generation (in terms of numbers). My objective here are to develop a more efficient laboratory rearing method of *T. nicklewoodi* and to test nematode release ability to prevent the establishment of populations of *F. occidentalis* on caged impatiens bedding plants in greenhouses.

Materials and Methods

Nematode Rearing

Transmission arenas were made by first creating a "thrips-egg-embedded bean leaf" by exposing the leaf to adult female thrips for 4 days in a plastic box at 24°C, 50-60% RH, and 14L: 10D photoperiod in a growth chamber for thrips oviposition. These physical environmental conditions were used for all subsequent procedures. Petioles were inserted in water-soaked cotton pads in plastic containers as described earlier. First instar larvae began to emerge from eggs in the rolled-leaf on same day and these were ready to be parasitized by nematodes. Four parasitized adult

female thrips, which defecate parasitic stage of the nematode, were used as the nematode source for propagation of the nematode colony (**Step 1** in Fig. 16). Each box with thrips eggs in a rolled leaf plus parasitized adult thrips was considered as a production unit. The units were incubated for 15 days in the growth chamber and then emerged adult female thrips were isolated in micropipette tips (200 µl capacity) (**Step 2 and 3** in Fig. 16). Thrips were recognized as parasitized by observing free living nematodes defecated from the host on the inside surfaces of these tips under a dissecting microscope. The opening of each tip was filled with paraffin to prevent thrips from escaping, and this held 50% honey solution in the middle of tip as food source for thrips (**Step 3** in Fig. 16). The parasitized thrips seen each day in this step were then used for transmission in the next generation of thrips (**Step 4** in Fig. 16) or greenhouse release trial in below. The thrips in the tip arena can survive up to 7 days without additional food sources.

Greenhouse Nematode Releases

This experiment was conducted from 3 November 2002 to 4 March 2003 in a plastic hoop greenhouse (9.5 W × 30.0 L × 3.7 H m) on the campus of the University of Massachusetts, Amherst, MA. Forty wooden frame cages (33 W × 60 L × 33 H cm) were constructed as described in Chapter III. A total of 40 impatiens (*Impatiens walleriana* Hook. f., var. 'Super Elfin White') trays were prepared by transplanting 48 impatiens plugs grown from seeds into each tray. One impatiens tray was put in each cage and the impatiens plants started to produce flower buds 17 days after the

transplanting. On 14 December 2002 when each cage had at least 5 flowers, half of the flowers in each cage were collected and placed in 0.03% dishwashing detergent solution to assess the density of pre-existing thrips. In ten of 40 cages, one or more thrips were found (all adult thrips were identified as *F. occidentalis*). However I decided not to exclude preexisting thrips population, but rather I assigned the ten infested cages randomly to four treatments, which were 'low nematode', 'high nematode', 'Spinosad (Conserve®, Dow AgroSciences LLC, Indianapolis, IN)', and a no-treatment control. Random assignment of additional 30 cages followed. After the assignment of cages to the four treatments, the mean number of each life stage of thrips in each cage was calculated by multiplying the number of thrips per flower with total number of flowers counted. After confirming that there was no statistical difference in thrips densities among the treatments in ANOVA (1st instar: $F_{3, 39} = 0.42$, $P = 0.741$; 2nd instar: $F_{3, 39} = 0.18$, $P = 0.910$; adult female: $F_{3, 39} = 0.67$, $P = 0.577$; adult male: $F_{3, 39} = 1.00$, $P = 0.404$), three female and three male thrips were released into each cage on 15 December 2002 to boost population growth.

I released parasitized female thrips as the source of nematodes into the 'low nematode' and 'high nematode' treatment cages 0, 6, 12, 13, 19, 34, and 43 days after the release of the healthy thrips. A total of seven parasitized thrips were released per cage in the 'low nematode' treatment and 14 in the 'high nematode' treatment. In 'low nematode' treatment cages, one parasitized thrips was released on each release day but, in the 'high nematode' treatment cages, two parasitized thrips were released on each release day.

In the 'Spinosad' treatment cages, I sprayed Conserve® (Dow AgroSciences LLC, Indianapolis, IN) twice, 7 and 14 days after the release of the healthy thrips, at the recommended rate in label. I sprayed each cage with 50 ml of a solution containing 0.027 ml a.i./ml.

Sampling Methods and Statistical Analysis

The first samples were collected on 9 January 2003, 6 days after 5th release of parasitized thrips, and subsequent samplings followed every 100 degree days (DD). Temperatures were recorded using three Hobo H8 data loggers (Onset Computer Corporation, Bourne, MA) placed inside of three randomly selected cages. Daily 24 h mean temperatures were calculated from each instrument and averaged to obtain the daily mean temperature values used to calculate the number of DD per day using the 10 °C lower limit.

Over the whole experiment, samples were taken six times. On each date, I first counted the total number of flowers in each cage and, then, picked one fourth of the flowers to estimate the number of thrips per flower and number of thrips per cage (in the flower niche). Flowers were placed in a 0.03% dishwashing detergent solution in a plastic container (148 ml capacity, TS5, Solo, Urbana, Illinois). The treatment and cage numbers of all samples were marked on the lids of the containers, which were taken to the laboratory for counting and identification of the thrips. Petals and sepals detached from the flowers in the detergent solution. Floating flower parts were removed one by one using tweezers, swirling them first to leave thrips behind. The

containers were then held for 5 min to let thrips sink. Thrips on the bottom of each container were then removed with a micropipette under a dissecting microscope and placed on a glass slide for counting and identifying thrips (Mound and Kibby, 1998). Nematode parasitization of thrips was confirmed by dissecting the thrips. Because all adults thrips recovered during whole experimental period were *F. occidentalis*, I assumed all immature thrips that I collected were also western flower thrips.

The number of thrips per cage (in the flower niche) was calculated by multiplying the number of thrips per flower per cage by the number of flowers per cage. This was done for four subgroups, i.e., 1st instar, 2nd instar, adult female, and adult male. Both the number of thrips per flower and number of thrips per cage (in the flower niche) as well as the number of flowers on each sampling date were analyzed by ANOVA using GLM procedure of SAS (SAS Institute, 1995) to identify significant effects of different levels of nematode introduction and insecticide on densities of healthy thrips populations. If significance in ANOVA was detected, data were further analyzed using Tukey's studentized range honestly significant difference (HSD) test for multiple comparisons (SAS Institute, 1995).

Sex ratios (proportion male) of healthy thrips were obtained in each cage by dividing the number of adult males by the sum of adult males and females. The sex ratios among treatments were analyzed by ANOVA using GLM procedure of SAS (SAS Institute, 1995) after arcsine transformation (Zar, 1996) to identify significant effects of different treatment on sex ratio of the healthy thrips population. Any significant difference between treatments was identified by Tukey's studentized range

HSD test after the ANOVA. Percent parasitism rate between the 'low nematode' and 'high nematode' treatments were compared using a *t* test, after arcsine transformation (Zar, 1996).

Results

Nematode Rearing

For the first 14 trials of the rearing method from October to December 2001, yields of F_1 parasitized female thrips was 3.9 times that of the P_1 thrips used for transmission (Table 11). However, in the last 20 trials, both the production of adult female thrips from the egg-embedded leaf and parasitized-thrips production ratios show decreases from 42.3 to 21.6 and from 3.9 to 2.3, respectively (Table 11). For a total of 15 months of rearing periods, the nematode-parasitized thrips were obtained 2.7 times of input and mean parasitism rate was 37%.

Greenhouse Release of Nematodes

Trends in two thrips densities, number of thrips per cage (in the flower niche) and number of thrips per flower, by life stage and treatment are given in Figs. 17 and 18. The best thrips control was achieved in the 'Spinosad' treatment. The number of thrips per cage in the 'Spinosad' treatment never reached one on any sampling DD for any life stages except 2nd instar larvae samples on 600 DD (Fig. 17) and none of the

four thrips life stages were found at levels higher than 0.09 thrips per flower (Fig. 18). The number of flowers in the 'Spinosad' treatment remained highest among other treatments, especially during last three sampling dates, and there was more than three times of flowers on 600 DD ($F_{3, 39} = 12.26$, $P < 0.001$) compared to control cages (Fig. 20).

In nematode treatment cages, neither the number of 1st instar larvae per cage nor per flower was significantly different from that in control, even though they always remained lower than number of 1st instars in control cages (except for number per cage on 100 and 600 DD) (Fig. 17 and 18). However, compared to control cages, both the number of 2nd instar larvae per cage and per flower in nematode treatment cages were significantly reduced on 400 DD only ($F_{3, 39} = 14.29$, $P < 0.001$) and 300 ($F_{3, 39} = 11.95$, $P < 0.001$), 400 ($F_{3, 39} = 16.29$, $P < 0.001$), and 500 ($F_{3, 39} = 8.22$, $P < 0.001$) DD, respectively (Fig. 17 and 18). In the adult stage, both the number per cage and the number per flower in the nematode treatment cages were significantly lower than in the no treatment control on 500 DD (female per cage: $F_{3, 39} = 7.46$, $P < 0.001$; male per cage: $F_{3, 39} = 11.69$, $P < 0.001$; female per flower: $F_{3, 39} = 7.53$, $P < 0.001$; male per flower: $F_{3, 39} = 14.40$, $P < 0.001$) (Fig. 17 and 18). Significant difference was also found in number of adult female thrips per flower on 400 DD between the nematode treatment cages and no treatment control cages ($F_{3, 39} = 5.95$, $P = 0.002$). No differences in either number per cage or number per flower, however, were found between 'low nematode' and 'high nematode' treatments on any DD of any life stages from Tukey's studentized range HSD test. Reductions of flowers in nematode

treatment cages were found on 400 DD ($F_{3,39} = 4.18$, $P = 0.012$) and 500 DD ($F_{3,39} = 8.38$, $P < 0.001$) only between 'low nematode' treatment and no treatment control cages.

Parasitism in larvae thrips remained low in both 'low nematode' and 'high nematode' treatments (Fig. 19). The highest nematode parasitism found in 1st instar was 4% (in 'high nematode' treatment on 100 DD) and in 2nd instar 7% ('low nematode' treatment on 200 DD). The adult male thrips were also found to have low parasitism rates throughout the sampling periods, except on 100 DD when the parasitism in 'low nematode' treatment reached 25% (Fig. 19). Adult female thrips had higher parasitism than larval thrips or adult male thrips during the sampling periods when parasitized thrips were released for thrips control. After 400 DD, nematode parasitism in adult female thrips decreased to under 15% (Fig. 19). Because I did not find any parasitized thrips in the no treatment control or the 'Spinosad' treatment cages, I analyzed the difference in parasitism rates between 'low nematode' and 'high nematode' treatments on each sampling date using *t* tests. A significant difference was found only in adult female thrips samples on 300 DD ($t = 2.75$; d.f. =13; $P = 0.017$), in which none of the adult female thrips in 'low' nematode treatment were found to be parasitized by the nematode.

Sex ratios of thrips populations were calculated in all treatments except in 'Spinosad' treatment where few adult thrips were collected. Sex ratio in control, 'low' nematode, and 'high' nematode treatment were all male-biased throughout the

sampling period and nematode treatment cages had higher proportion male than control cages on 200, 400, 500, and 600 DD (Fig. 19). Statistical differences, however, were not found on any of the sampling date.

Discussion

The basic procedure used for nematode rearing followed previous methods, which released parasitized adult female *F. occidentalis* (as a source of nematode) into 50 1st instar larvae in two different transmission arenas, a rolled bean leaf (Chapter I) or microcentrifuge tubes (Arthurs and Heinz, 2002) for parasitization of healthy larval thrips. The rearing method for *T. nicklewoodi* presented here is more efficient than that of Arthurs and Heinz (2002) because, first, production of parasitized thrips was increased by up to 95% compared to Arthurs and Heinz (2002) (from 2.0 to 3.9 output/input ratio) and, second, the number of steps in the rearing process was reduced by 50% (from 8 to 4 steps), including elimination of two major time-consuming steps in the method of Arthurs and Heinz (2002), i.e., isolating and transferring thrips between microcentrifuge tubes. Isolating thrips from tubes to watch for nematode excreted from the thrips is not necessary in my method because nematodes on the inside walls the pipette tips can be directly observed under the dissecting microscope. In addition, aspirating thrips into the transmission arena and transferring the thrips from the transmission arena to the rearing boxes is replaced by one step, preparing thrips egg-embedded rolled leaves, in my method. Unlike in the method of Arthurs and Heinz (2002), I did not recycle the parasitized thrips used as nematode source.

Greater output was achieved by leaving them alone in the box, probably due to increased exposure time for parasitization. In addition, I found the recycling of the parasitized thrips reduced the production of parasitized thrips.

However, a loss of efficiency (production ratio, output/input) due to inbreeding depression of both thrips and nematode (Table 11) and high production cost in the present method employing live host may hinder use of *T. nicklewoodi* for augmentative releases for thrips control in commercial greenhouses. The frequent introduction of field-collected thrips or nematodes into a laboratory colony or development of *in vitro* rearing method would be necessary to increase the efficiency of nematode production.

In the nematode release trial, *T. nicklewoodi* did reduce thrips population on the impatiens flowers by up to 44% for second instar on 400 DD, 68% for adult female on 500 DD, 49% for adult male on 500 DD compared to thrips numbers in the untreated control (Fig. 17 and 18). The degree of reduction of adult thrips was less than the 79% reduction (versus control) of the adult stage seen in previous study (Chapter III) and less than the 77% control of the pupal stage achieved with another nematode, *Steinernema carpocapsae* (Helyer *et al.*, 1995). At the end of the experiment, the nematode parasitism in adult thrips had decreased, resulting in an increase in thrips population to nearly that of the control. The lack of persistence of nematode transmission and subsequent decrease in nematode parasitism might be caused by the higher proportion of male thrips throughout the experiment (Fig. 21). Because parasitized male thrips die quickly (Table 1), there might be less chance for nematode transmission.

Even though the number of flowers in nematode treatment cages after 300 DD was always higher than in the untreated control, the trend of change in number of flowers followed similar patterns, and flower production decreased as the thrips population density increased at the end of experiment. In conclusion, *T. nicklewoodi* released seven times on caged impatiens in a greenhouse did not provide preventative control of *F. occidentalis*, though the population growth of second instar, adult female, and male thrips was suppressed by 44, 68, and 49%, respectively.

Table 1. Effects of parasitization by *Thripinema nicklewoodi* on the longevity of adults of *Frankliniella occidentalis* at 24°C.

Adult WFT		n	Longevity ± SD (days)
Female	Healthy	76	15.8 ± 6.7
	Parasitized	46	11.7 ± 8.2
Male	Healthy	33	17.8 ± 8.9
	Parasitized	21	6.9 ± 6.1

Table 2. Effect of *Frankliniella occidentalis* life stages on parasitization rate by *Thripinema nicklewoodi*.

% Parasitism of WFT Life Stages (± 95% C.I., n = 30)					
1st instar	2nd instar	prepupa	pupa	adult male	adult female
63.3 ± 17.2	70.0 ± 16.4	63.3 ± 17.2	30.0 ± 16.4	26.7 ± 15.8	13.3 ± 12.2

Table 3. INSV infection rates in *Frankliniella occidentalis* exposed to nematode parasitization versus control thrips.

	Exposed to nematodes	Control	P (*t*-test)
Mean no. of adult thrips emerged from exposed larvae	31.7	27.8	0.280
Mean frequency of adult thrips positive by ELISA	0.222	0.321	0.156
For subgroup: parasitized thrips	0.150		0.017[a]
For subgroup: non-parasitized thrips	0.263		0.388[a]

[a]Mean frequency of subgroup was tested against the control group.

Table 4. Detection of INSV infection in *Frankliniella occidentalis* after nematode parasitization.

	Percentage of thrips positive for INSV		
Days after nematode exposure	Nematode-parasitized	Non-parasitized	P (Fisher's exact test)
0	30.0 (9/30)	43.3 (13/30)	0.301
5	43.3 (13/30)	50.0 (15/30)	0.617
10	53.3 (16/30)	76.7 (23/30)	0.066

Table 5. INSV transmission by nematode-parasitized thrips versus non-parasitized thrips.

	Percent of impatiens leaves	
	with symptoms	positive in ELISA
Nematode-parasitized thrips	18.8 (3/16)	37.5 (6/16)
Non-parasitized thrips	21.7 (5/23)	43.5 (10/23)
P (Fisher's exact test)	1.000	0.752

Table 6. Area (mm^2) of bean leaf damaged by feeding of nematode-parasitized versus non-parasitized female thrips in a standard assay.

	Adult females			Larval females[a]		
	Parasitized	Non-parasitized	P (*t*-test)	Parasitized	Non-parasitized	P (*t*-test)
Daily mean leaf damage (SD)	0.54 (0.37)	2.94 (1.92)	<0.001	2.14 (0.50)	2.31 (0.54)	0.280
Total leaf damage (SD)	-[b]	-[b]		11.91 (3.12)	12.51 (3.06)	0.514
n	24	30		22	24	

[a]Leaf damage was investigated from first through second instar larvae.

[b]Total leaf damage was not calculated because of the differential survivorship between nematode-parasitized and non-parasitized adult thrips.

Table 7. Honey feeding by nematode-parasitized versus non-parasitized adult female thrips.

	n	Feeding		Resting	
		Time (s)	Frequency	Time (s)	Frequency
Nematode-parasitized	17	451.2	14.4	1510.1	6.4
Non-parasitized	17	577.2	13.5	1133.4	6.4
P (*t*-test)		0.038	0.746	0.114	0.952

Table 8. Pollen feeding by nematode-parasitized versus non-parasitized adult thrips.

	Female thrips		Male thrips	
	Parasitized	Non-parasitized	Parasitized	Non-parasitized
Mean no. (SD) of pollen grains fed on	77.8 (23.7)	125.5 (63.6)	44.4 (22.4)	56.5 (22.7)
n	13	25	25	38

Table 9. Mean number (SD) of impatiens pots per cage added during sampling periods.

Sampling periods (DD)	Treatments			*P* (ANOVA)
	Control	Low	High	
0 – 200	0.0	0.0	0.0	-
200 – 400	0.0	0.0	0.0	-
400 – 600	0.0	0.0	0.0	-
600 – 800	1.3 (2.5) A	0.1 (0.3) A	1.1 (3.1) A	0.470
800 – 1000	7.6 (5.9) A	1.0 (2.0) B	2.4 (4.9) B	0.008
1000 – 1200	5.4 (3.0) A	1.9 (2.5) B	2.0 (2.4) B	0.009
1200 – 1400	2.6 (1.2) A	1.7 (1.3) A	1.6 (1.7) A	0.243

Table 10. Mean number (SD) of impatiens flowers per cage sampled.

Sampling date (DD)	Treatments			*P* (ANOVA)
	Control	Low	High	
0	3.3 (0.2) A	3.0 (0.2) A	3.3 (0.3) A	0.818
200	24.9 (1.3) A	24.2 (1.7) A	24.7 (1.7) A	0.949
400	64.4 (4.1) A	66.8 (3.0) A	71.6 (2.7) A	0.312
600	50.4 (4.9) A	54.8 (2.1) A	59.4 (3.8) A	0.263
800	29.5 (6.1) A	48.7 (2.8) B	47 (6.3) AB	0.030
1000	10.3 (2.4) A	21.0 (3.5) A	25.4 (7.0) A	0.086
1200	9.3 (0.9) A	17.7 (3.5) A	17.9 (4.8) A	0.153
1400	6.5 (0.7) A	10.7 (2.9) A	12.9 (3.6) A	0.256

Table 11. Production of nematode-parasitized adult female thrips using egg-embedded rolled bean leaf.

Rearing periods	n	Mean no. of adult female thrips collected (SD)	Parasitization rate (SD)	Parasitized thrips production ratio (output/input) (SD)
Oct 2001 – Dec 2001	14	42.3 (19.4)	0.38 (0.18)	3.9 (2.1)
Jan 2002 – Mar 2002	13	35.5 (13.4)	0.33 (0.15)	2.8 (1.2)
Apr 2002 – Jun 2002	3	41.0 (10.8)	0.44 (0.18)	4.3 (0.7)
July 2002 – Sep 2002	15	26.5 (13.8)	0.25 (0.14)	1.7 (1.2)
Oct 2002 – Dec 2002	20	21.6 (11.7)	0.46 (0.21)	2.3 (1.3)

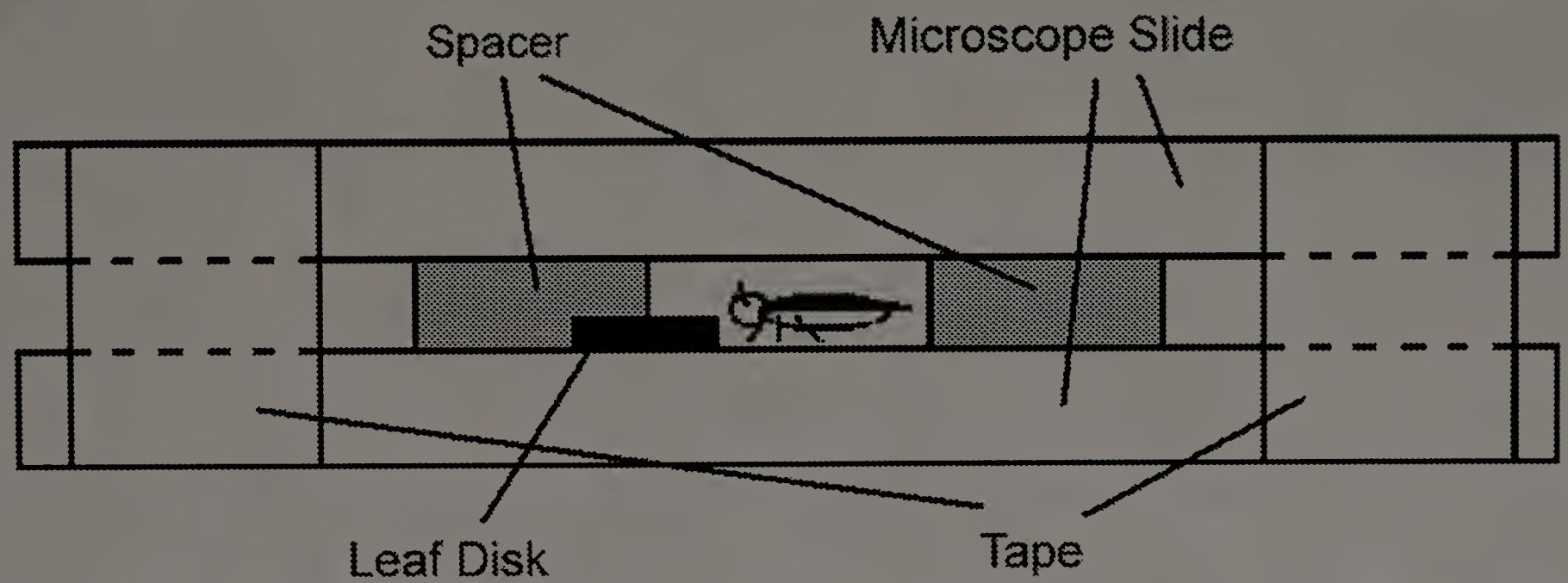

Figure 1. Diagram of microscope slide arena used to confine adults of *Frankliniella occidentalis* to detect excretion of nematodes.

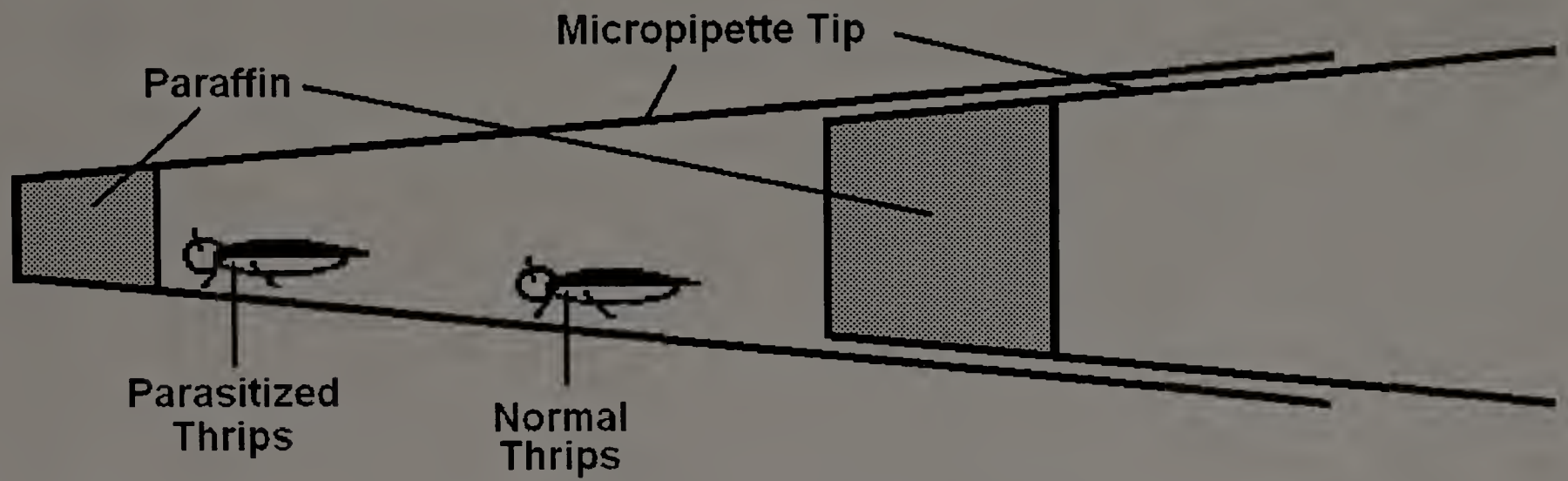

Figure 2. Diagram of micropipette tips used to confine *Frankliniella occidentalis* life stages for the host preference test.

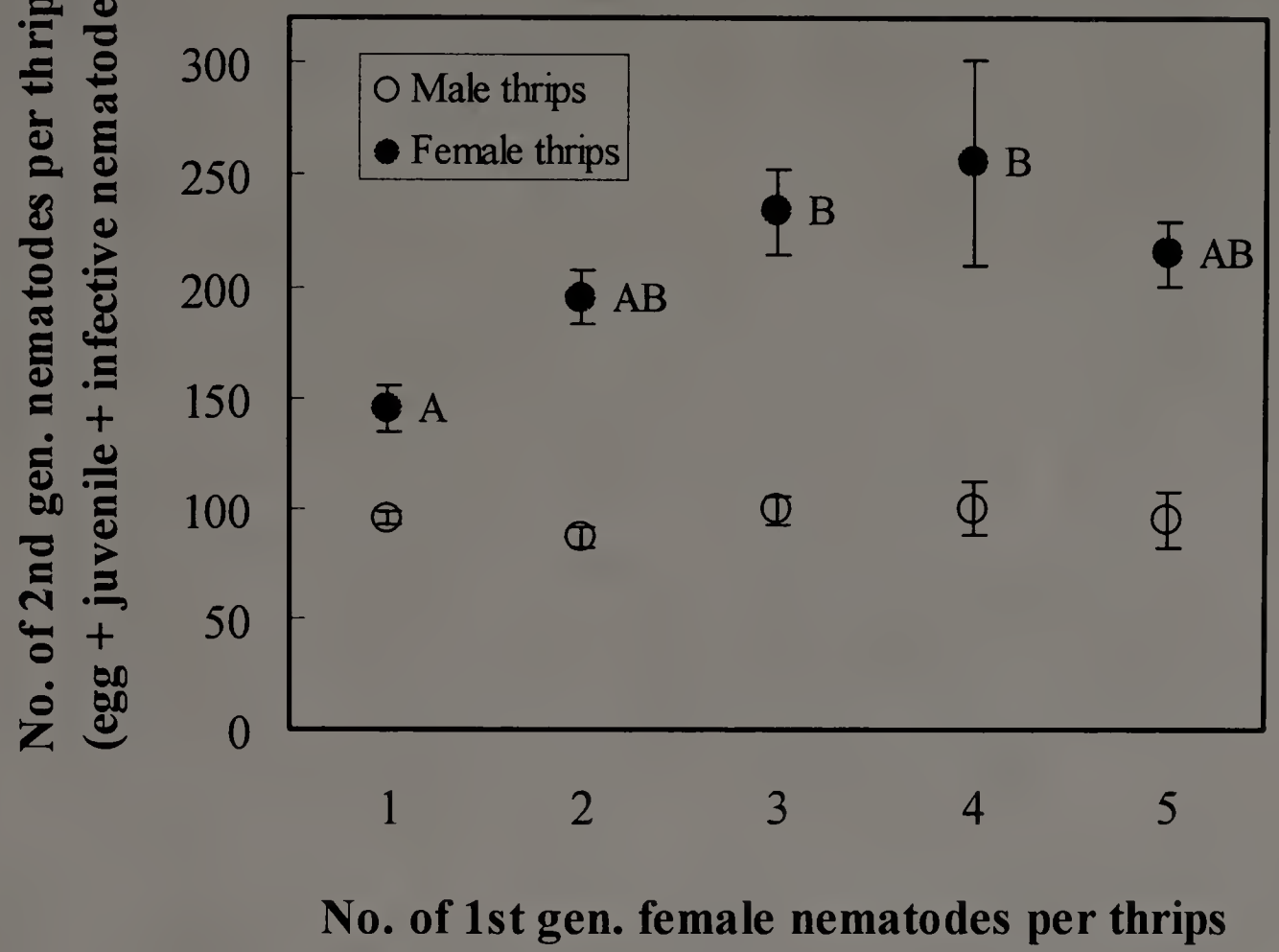

Figure 3. Mean numbers of second generation *Thripinema nicklewoodi* in dissected *Frankliniella occidentalis* adults in relation to host sex and the number of first generation female nematodes initially parasitizing the host. Means followed by the same letter are not significantly different, Tukey studentized range HSD test, $P = 0.05$. Vertical lines denote standard errors.

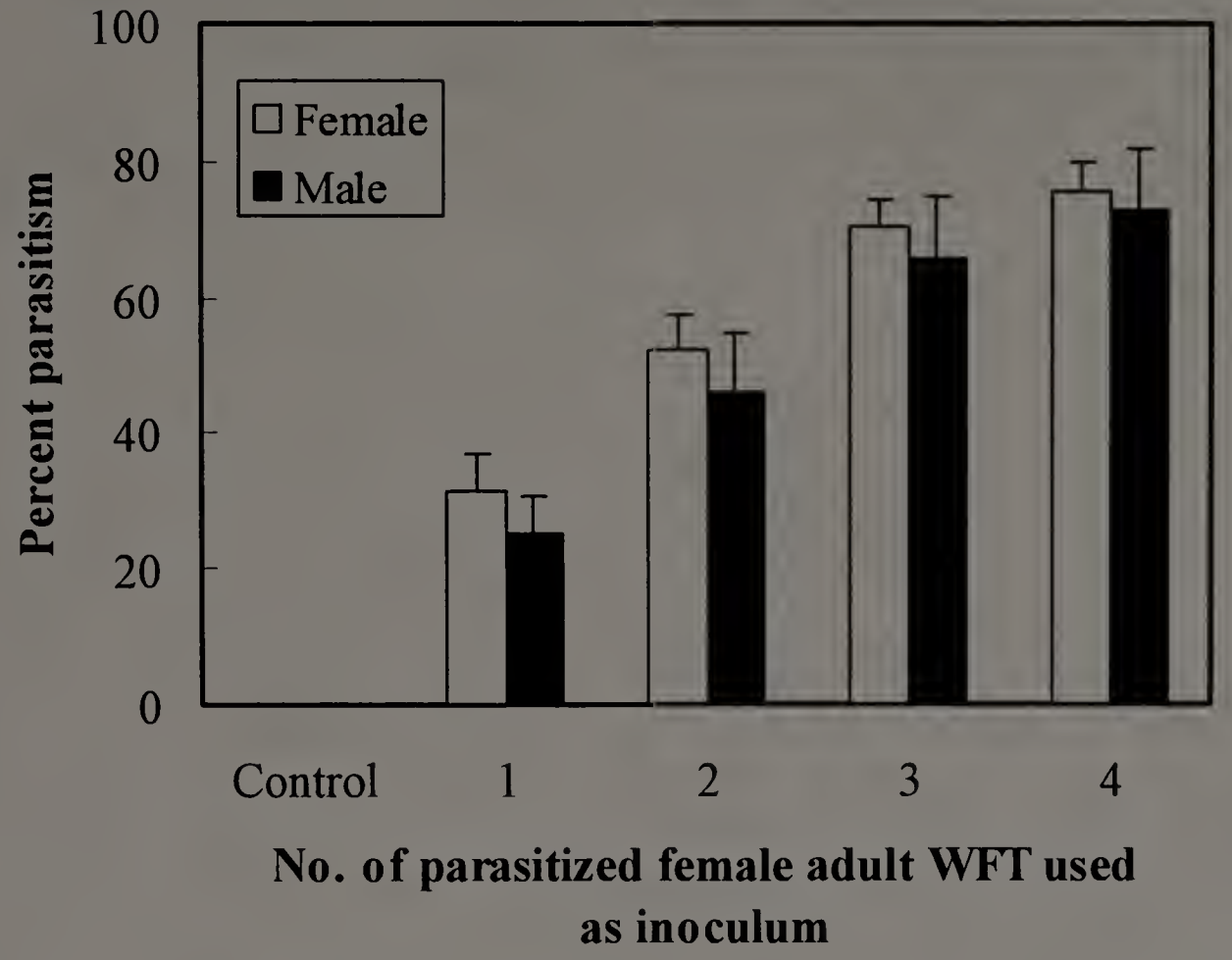

Figure 4. Effect of inoculum level (number parasitized adult *Frankliniella occidentalis*) on the parasitism rate of larval thrips by *Thripinema nicklewoodi*. Vertical lines denote standard errors.

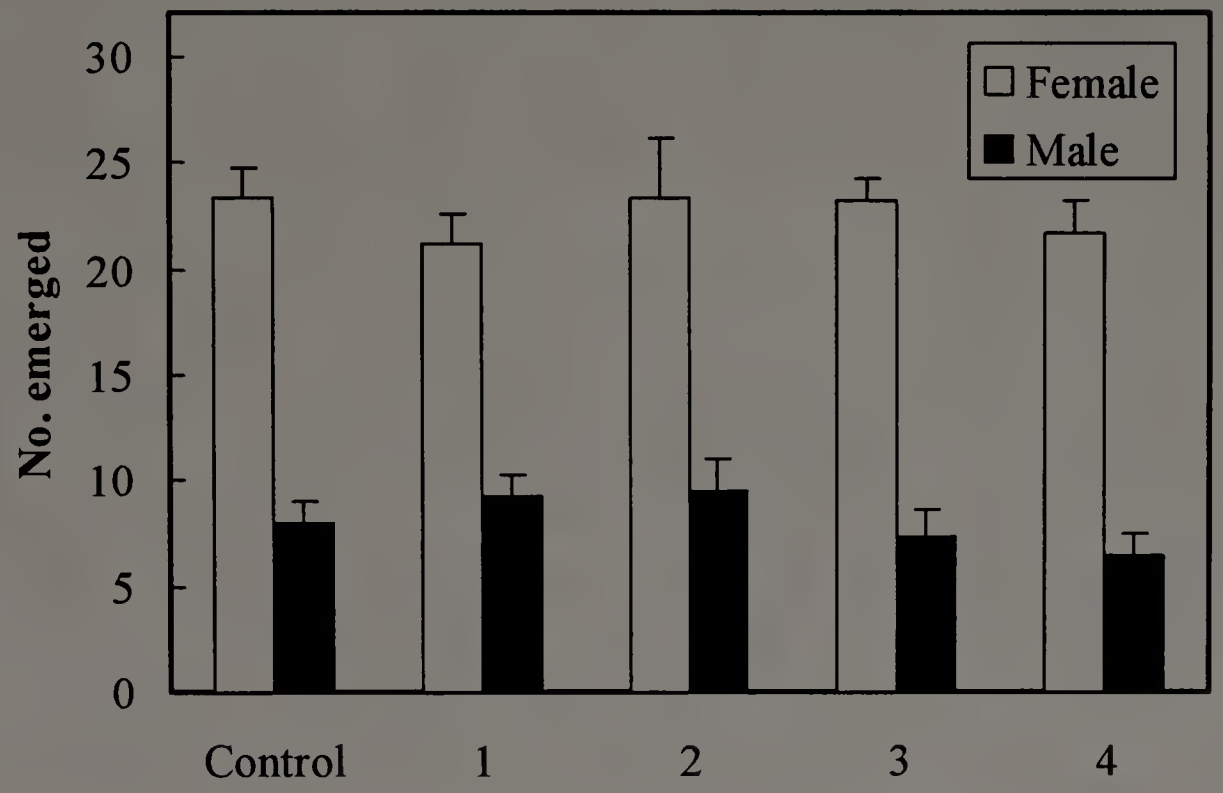

Figure 5. Effect of inoculum level (number parasitized adult *Frankliniella occidentalis*) on survival of larval thrips to the adult stage. Vertical lines denote standard errors.

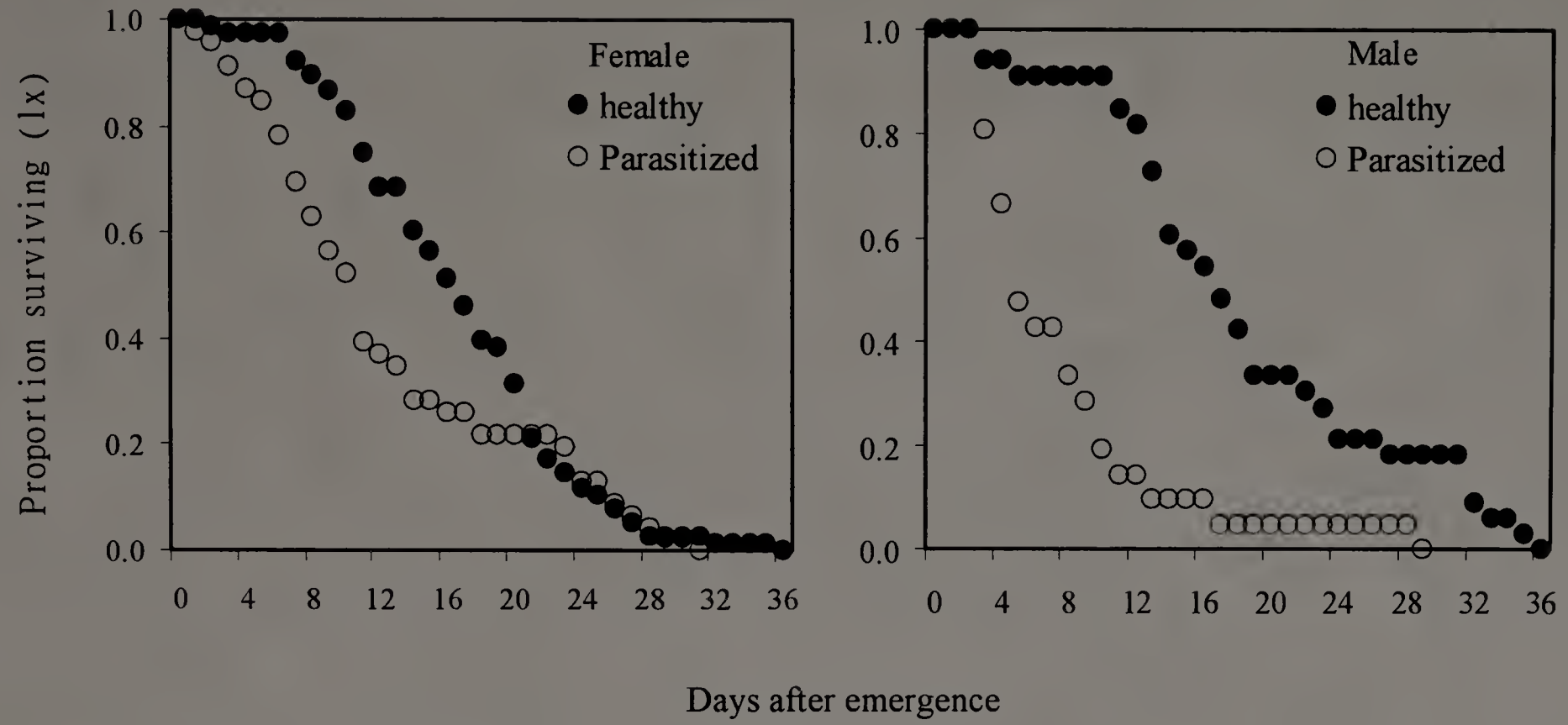

Figure 6. Survivorship of cohorts of male or female *Frankliniella occidentalis* adults that are either healthy or parasitized by *Thripinema nicklewoodi*.

Figure 7. *Thripinema nicklewoodi* escaping from *Frankliniella occidentalis* through anus.

Figure 8. *Thripinema nicklewoodi* escaping from *Frankliniella occidentalis* through anus.

Figure 9. *Thripinema nicklewoodi* penetrating *Frankliniella occidentalis* through abdominal intersegmental membrane.

Figure 10. *Thripinema nicklewoodi* penetrating *Frankliniella occidentalis* through coxal cavity.

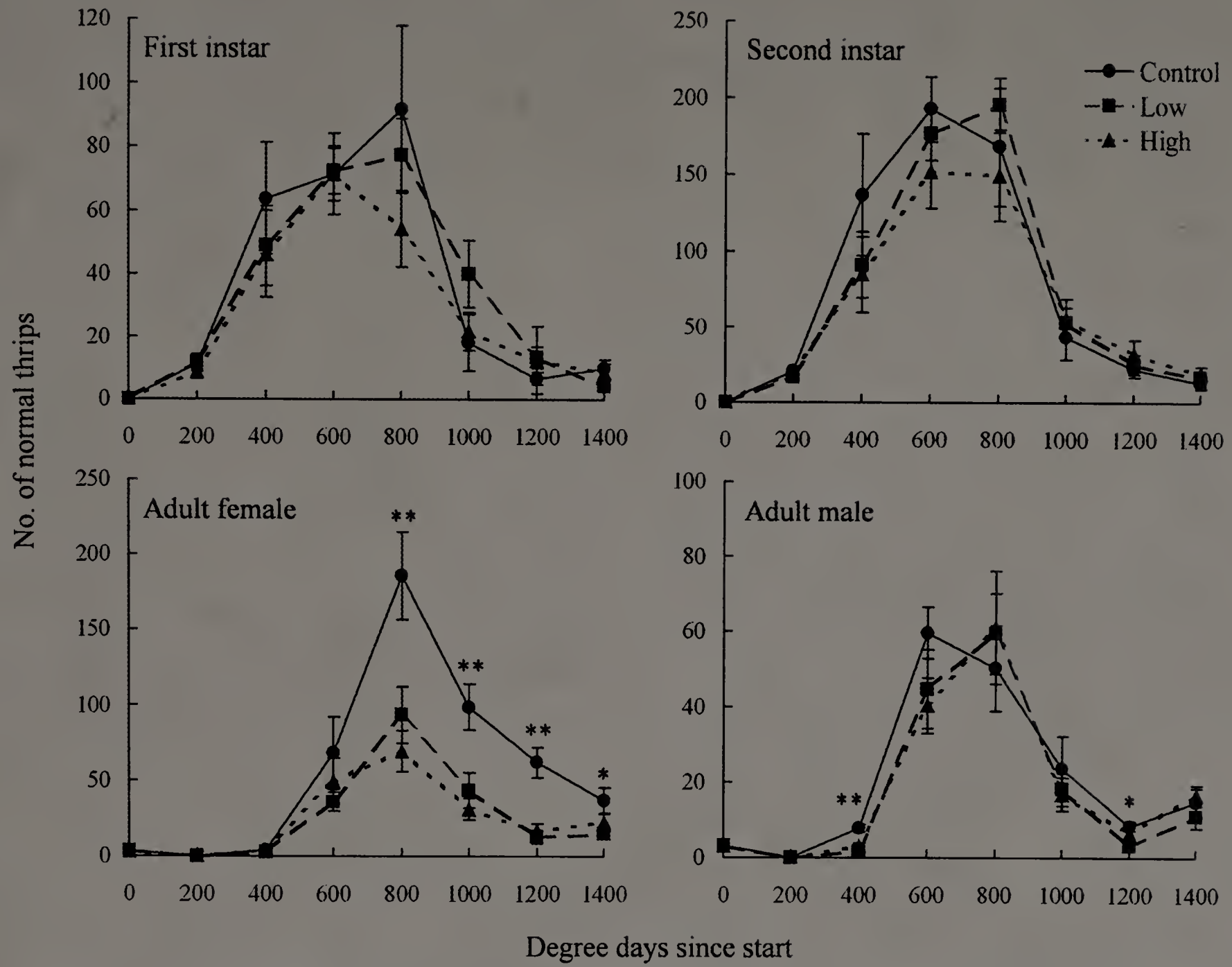

Figure 11. Change in density of healthy thrips in three experimental thrips populations with different initial rates of parasitism by *Thripinema nicklewoodi*. Significances among treatments in ANOVA are marked as * ($P < 0.05$) and ** ($P < 0.01$).

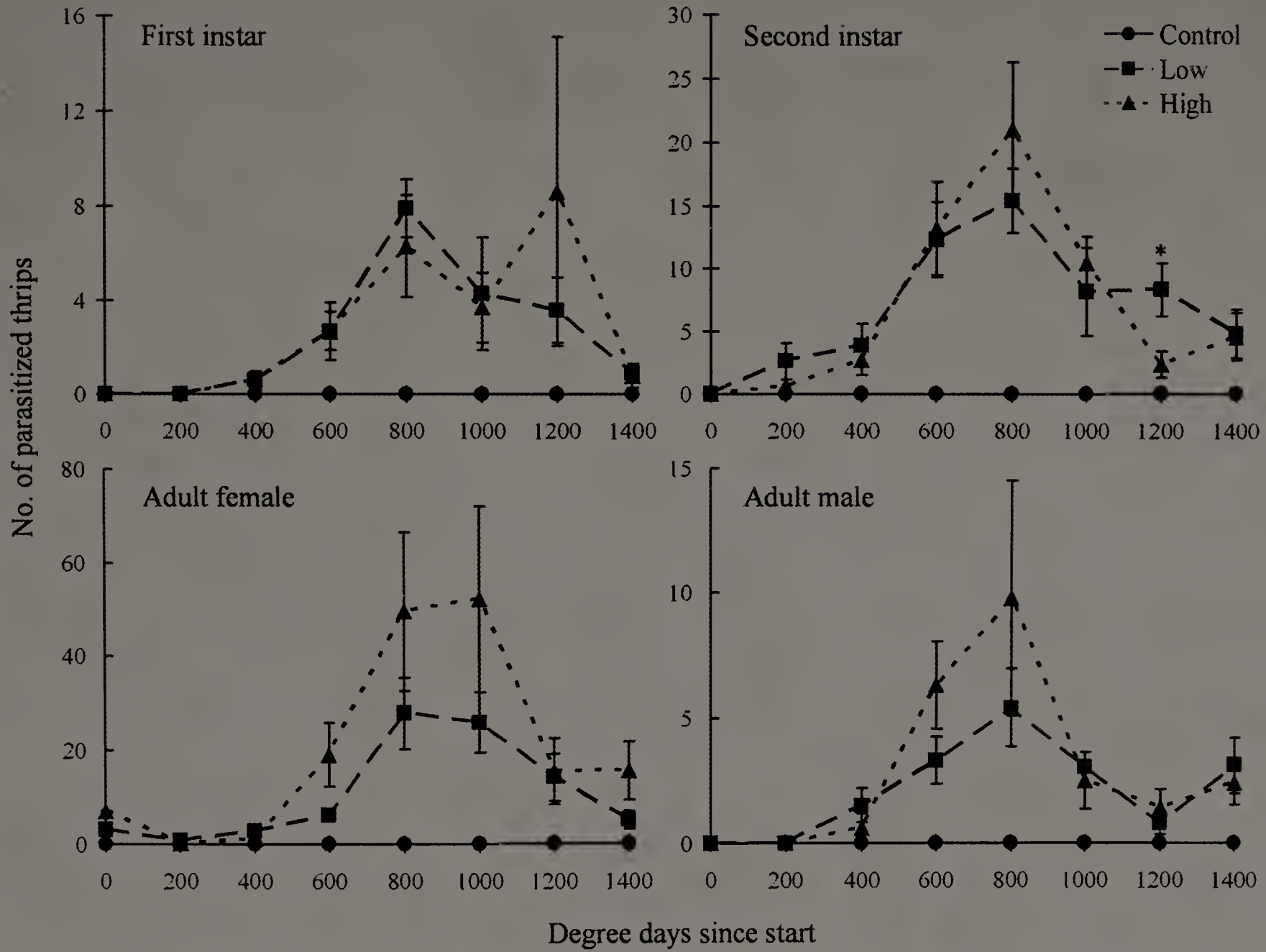

Figure 12. Numbers of parasitized thrips in three experimental thrips populations with different initial rates of parasitism by *Thripinema nicklewoodi*. Significant difference between treatment is marked as * ($P < 0.05$).

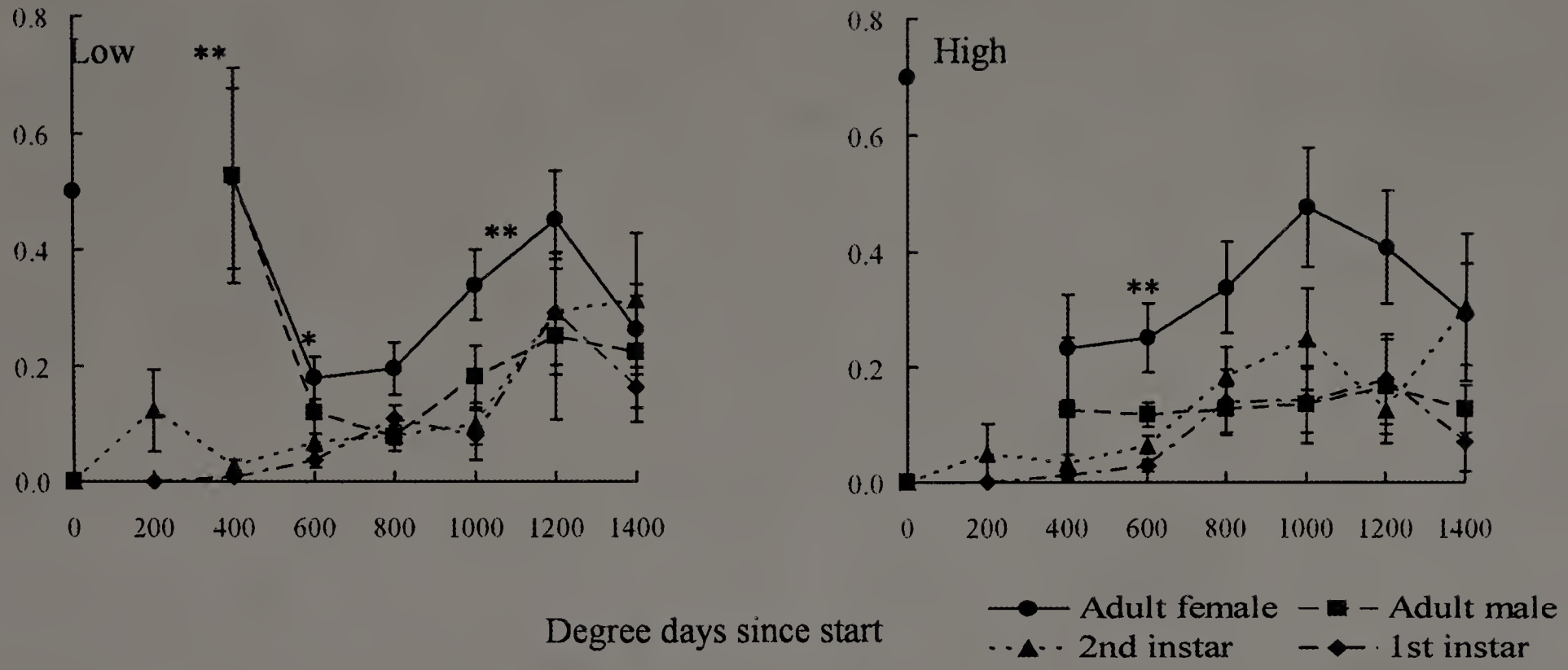

Figure 13. Trends in percentage parasitism for subpopulations in two experimental thrips populations with different initial rates of parasitism by the nematode. Significances among treatments in Kruskal-Wallis single factor analysis of variance by rank are marked as * ($P < 0.05$) and ** ($P < 0.01$), and vertical lines denote standard errors.

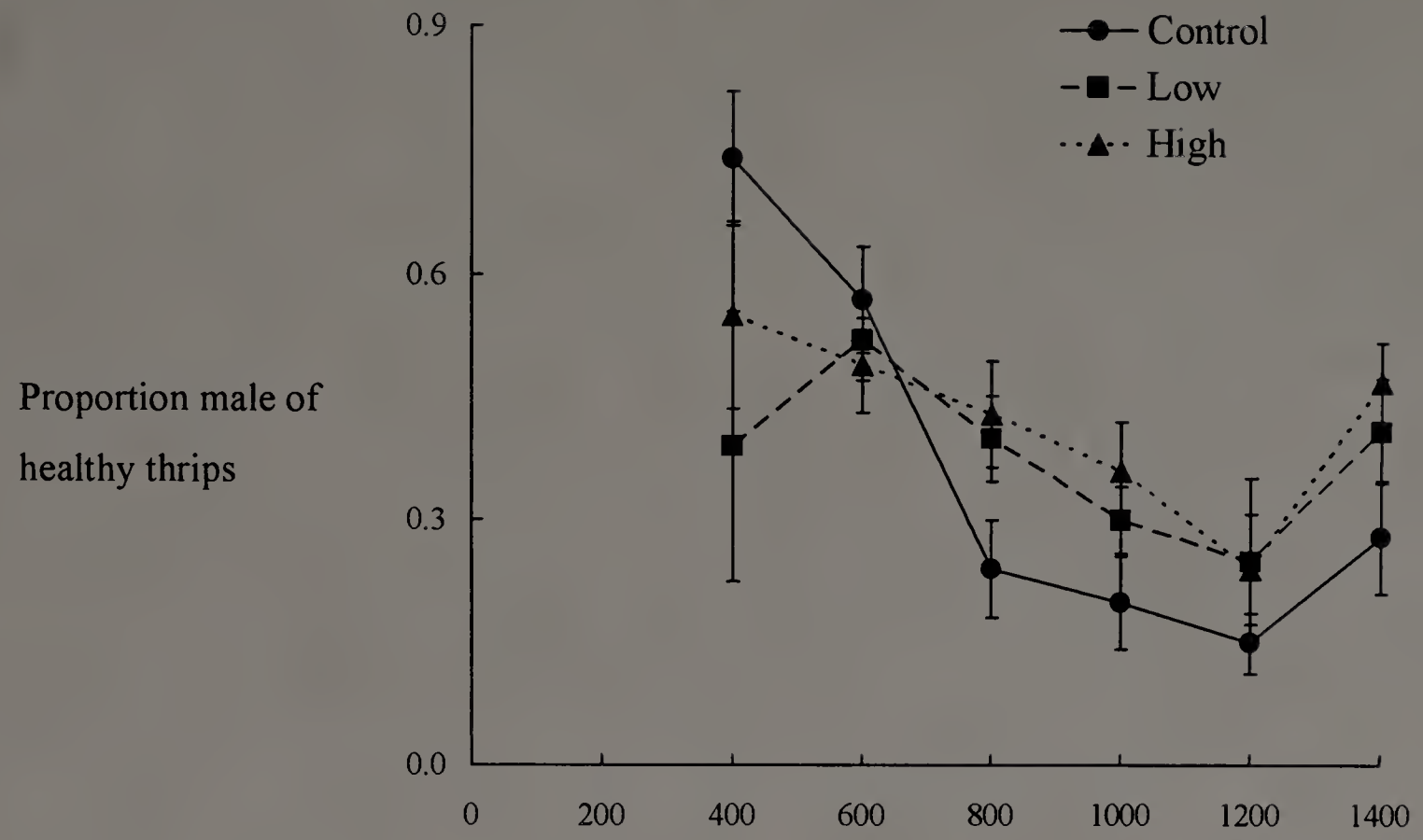

Figure 14. Sex-ratio dynamics of healthy thrips in three experimental thrips populations with different initial rates of parasitism by the nematode. Significance among treatment is marked as * ($P < 0.05$), and vertical lines denote standard errors.

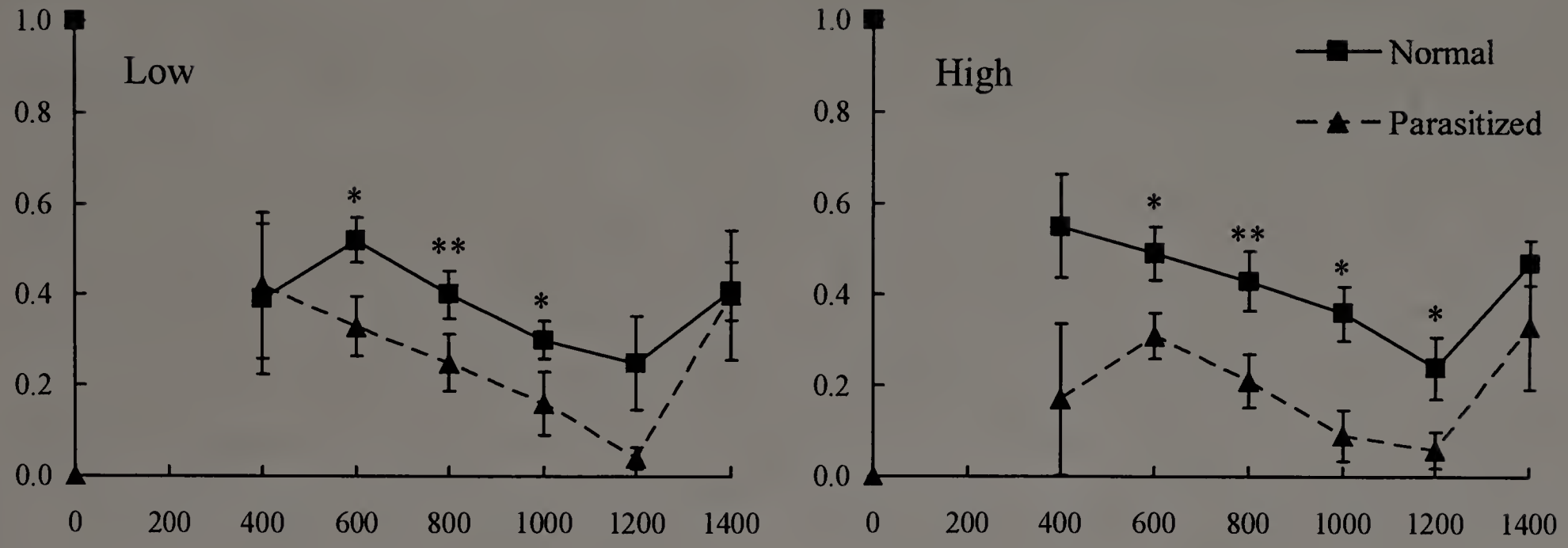

Figure 15. Sex-ratio dynamics of healthy and parasitized thrips in two experimental thrips populations with different initial rates of parasitism by the nematode. Proportion male was compared between healthy and parasitized thrips in both treatments. Significant differences between healthy and parasitized thrips are marked as * ($P < 0.05$) and ** ($P < 0.01$), and vertical lines denote standard errors.

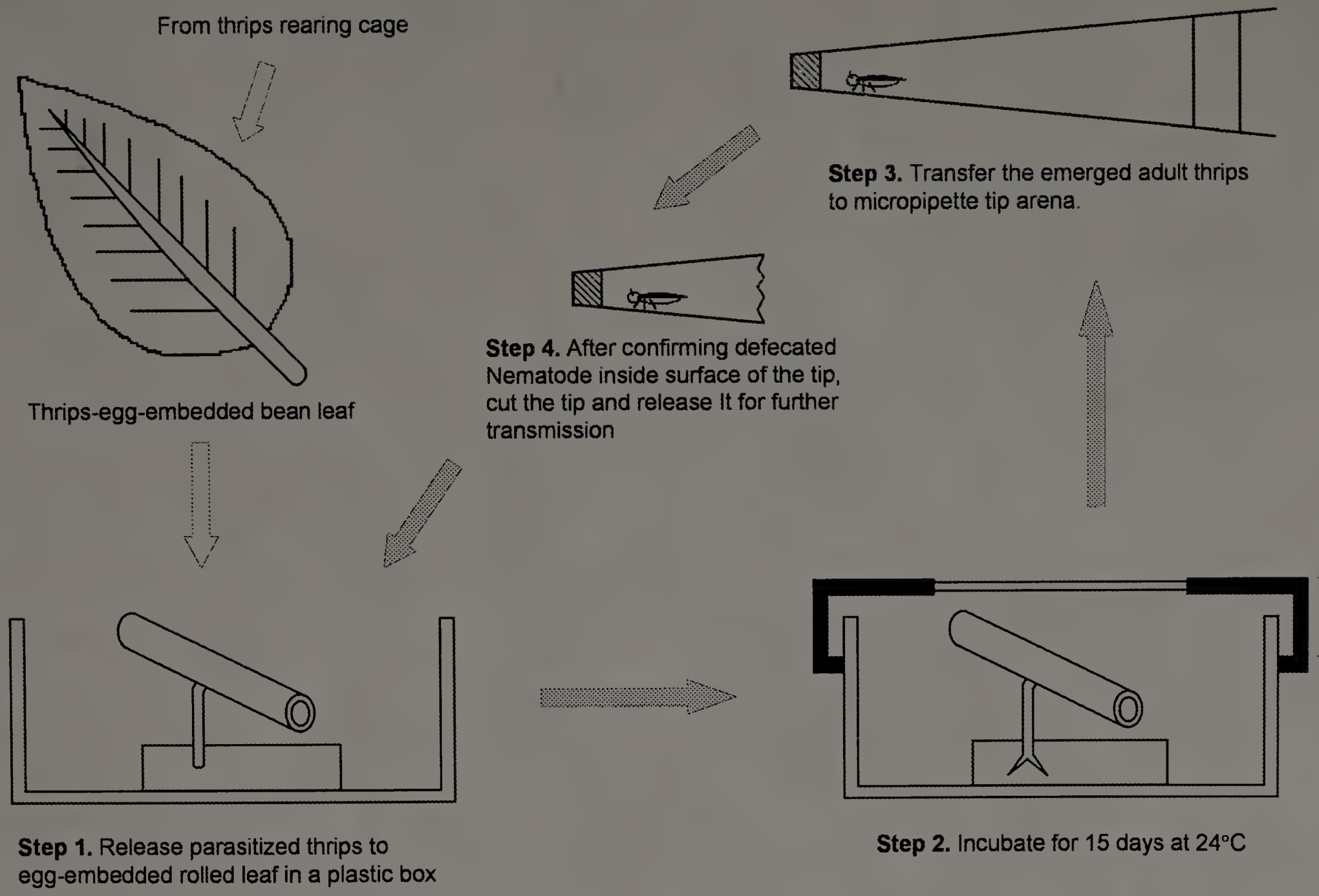

Figure 16. Diagram for *Thripinema nicklewoodi* rearing using thrips egg-embedded rolled bean leaf.

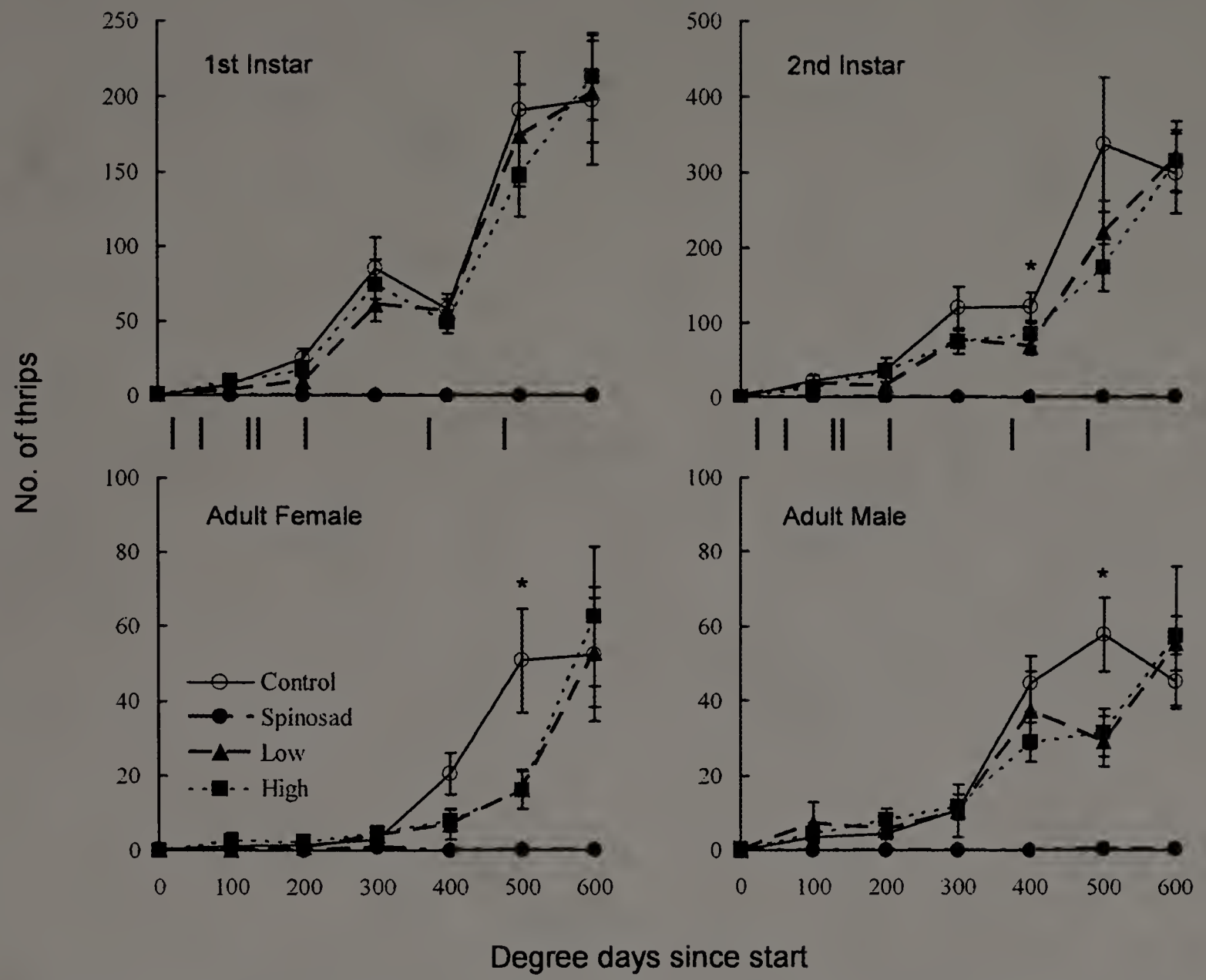

Figure 17. Number of thrips per cage (in the flower niche) in four treatments. Significance between control and any of two nematode treatments from Tukey studentized range HSD test marked as * ($P < 0.05$), and vertical lines denote standard errors. Vertical bars under X axis indicate degree day when the nematode released.

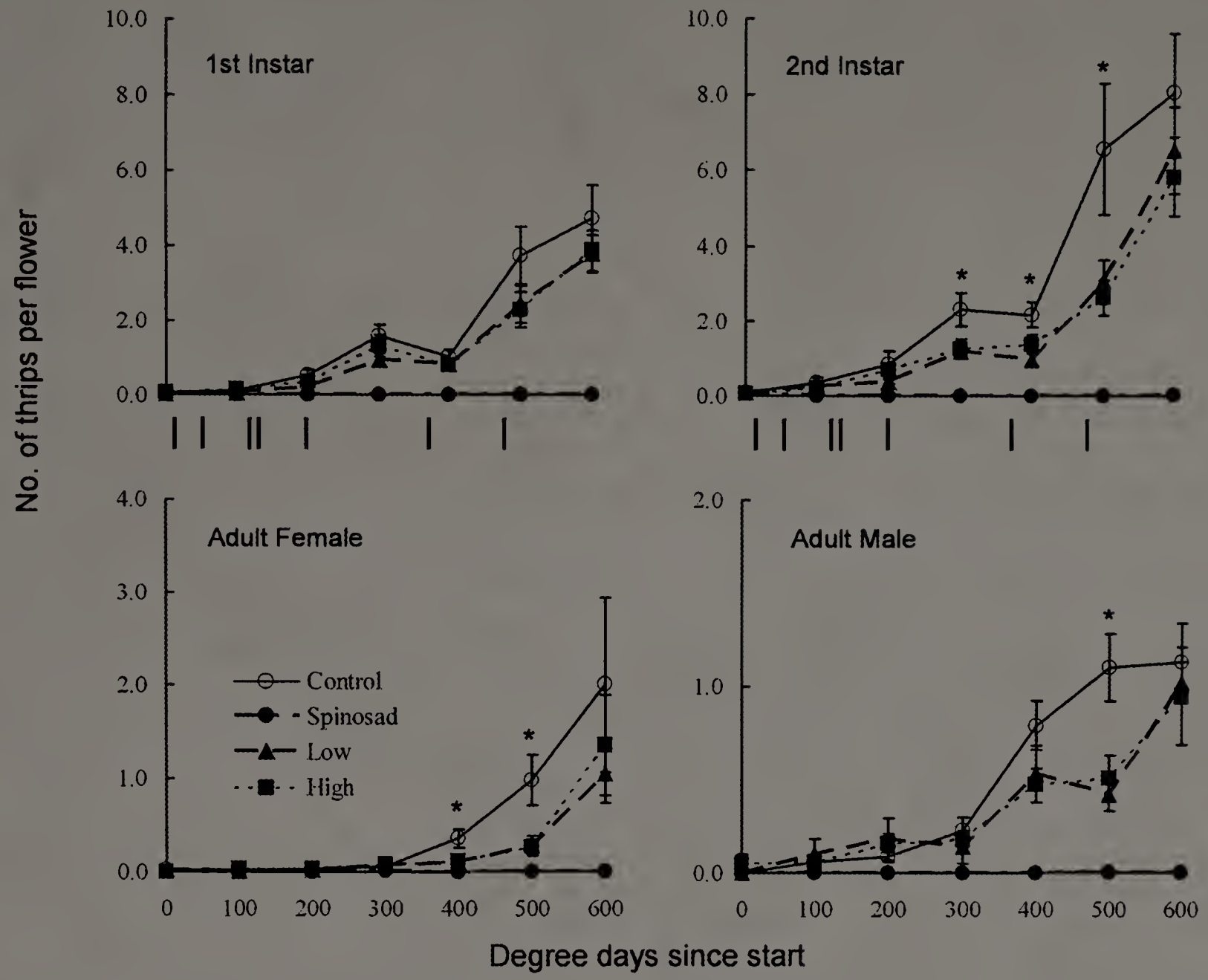

Figure 18. Number of thrips per flower in four treatments. Significance between control and any of two nematode treatments from Tukey studentized range HSD test marked as * ($P < 0.05$), and vertical lines denote standard errors. Vertical bars under X axis indicate degree day when the nematode released.

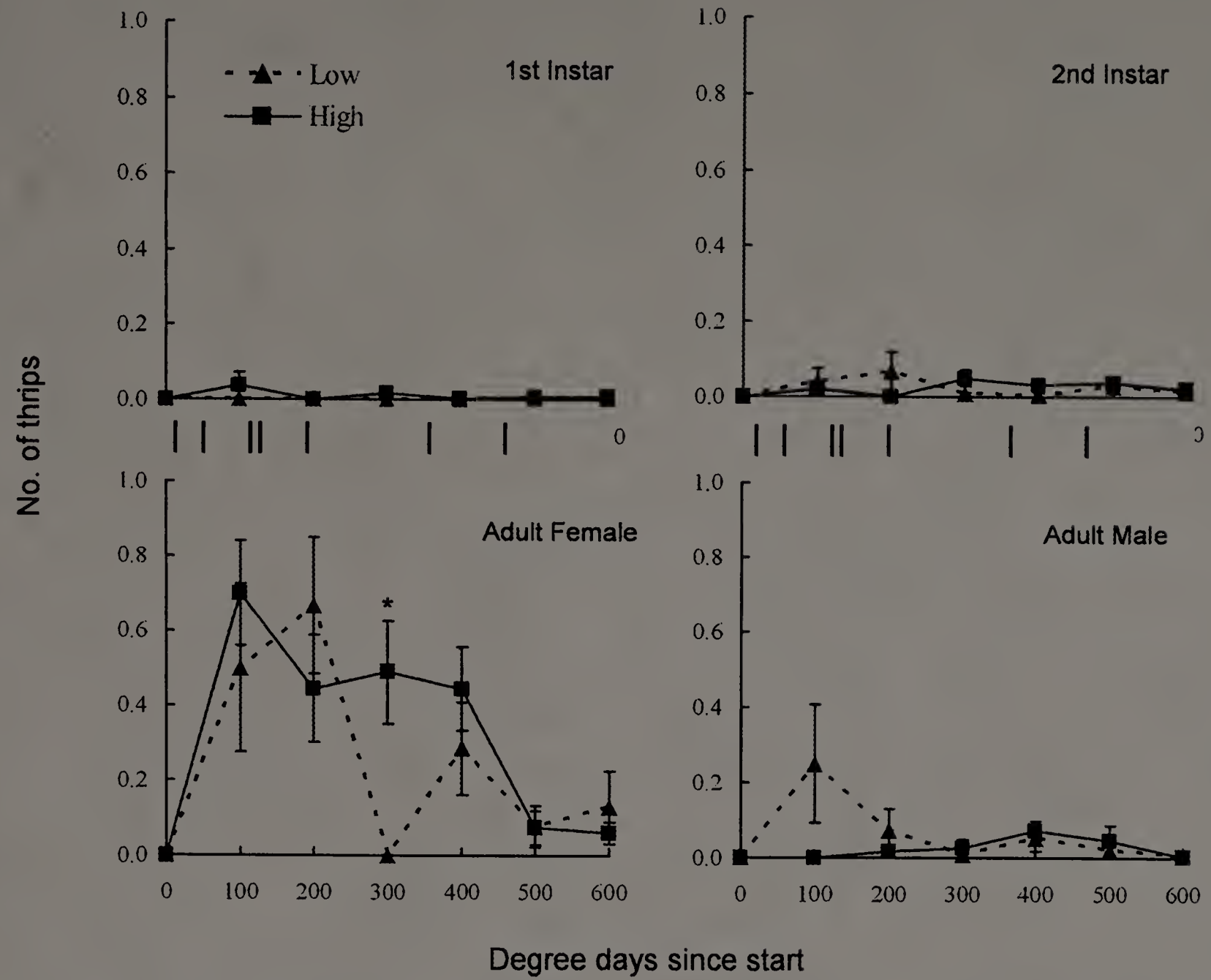

Figure 19. Dynamics of parasitism rates in both 'Low nematode' and 'High nematode' treatments. Significance among treatment from *t* test is marked as * ($P < 0.05$), and vertical lines denote standard errors.
Vertical bars under X axis indicate degree day when the nematode released.

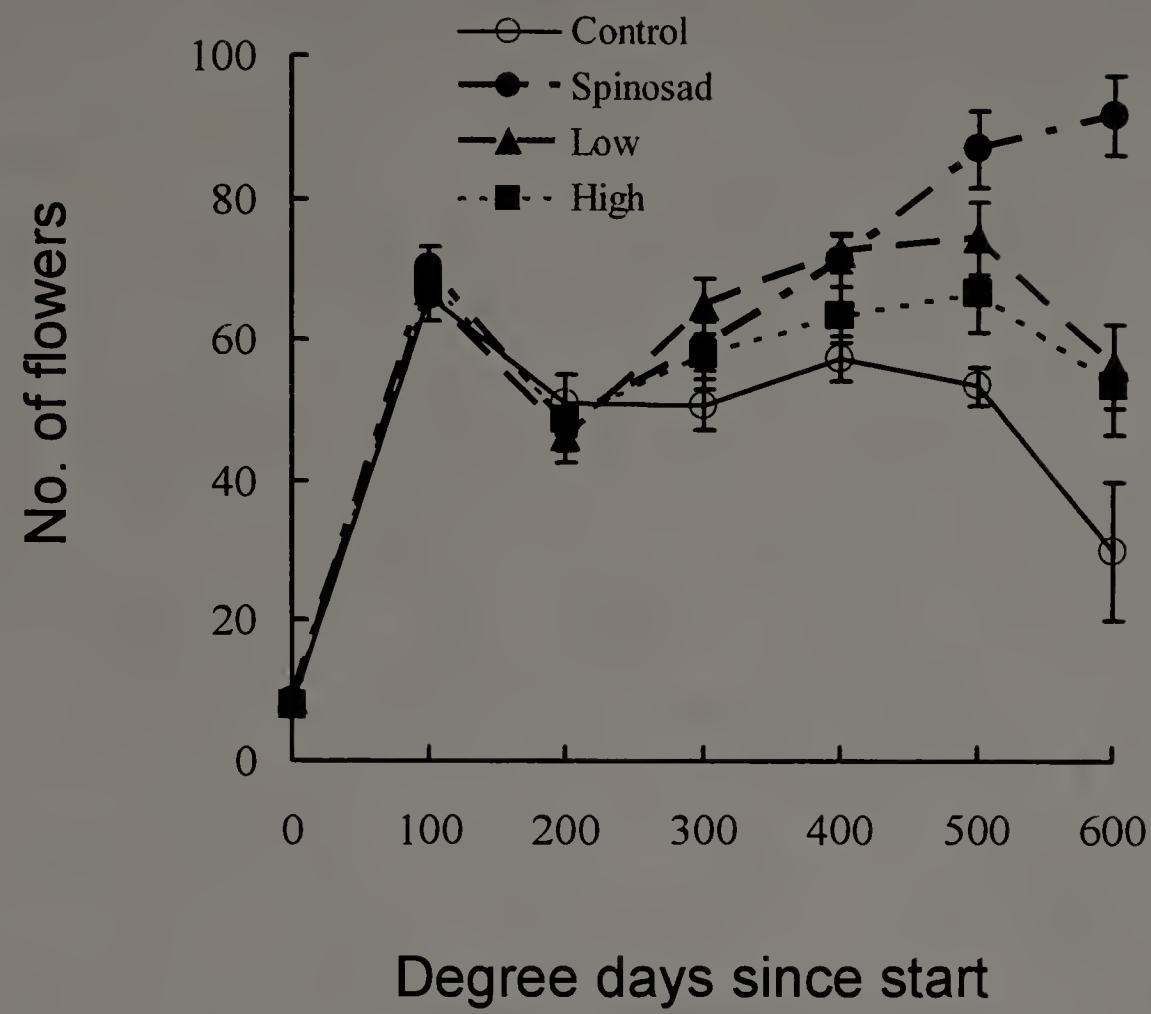

Figure 20. Trends in number of flowers among the four treatments. Numbers with on each DD followed by the different letter were significantly different in a Tukey's studentized range HSD test ($P < 0.05$), and vertical lines denote standard errors.

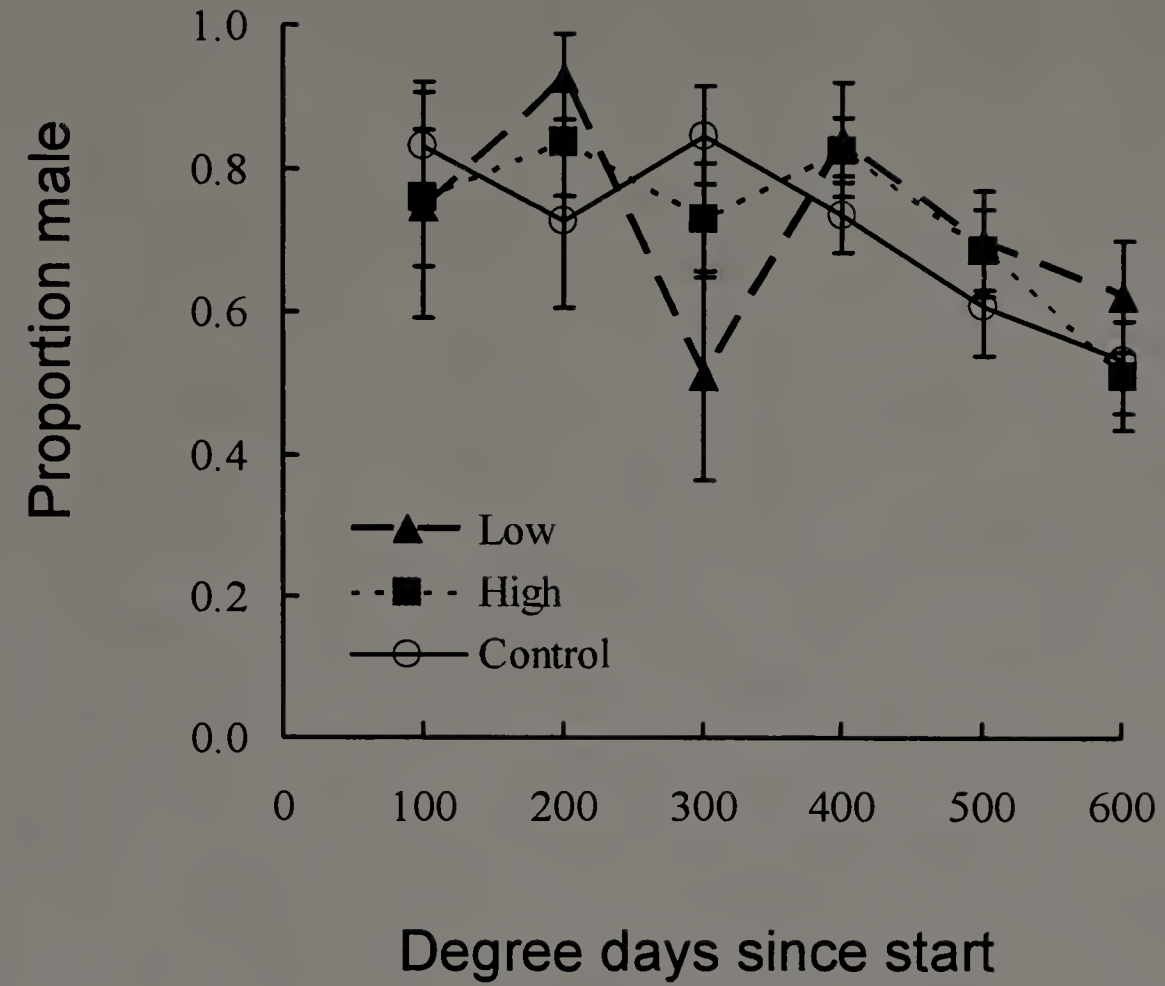

Fig. 21. Sex-ratio (proportion male) of thrips in three treatments. No statistical significance was found on any sampling date from ANOVA, and vertical lines denote standard errors.

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